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[54]发明名称 用近红外光谱测定目标中葡萄糖浓度的方法

[57]摘要

活组织中的葡萄糖浓度作为目标是通过下述方法测定的。将近红外辐射投射到活组织上，接收从该活组织散射所产生的辐射。对所产生的辐射进行光谱分析，从具有葡萄糖分子 OH 基团的吸收峰的一个波长区检测一个第一吸收信号，从具有活组织中的 NH 基团吸收峰的波长区检测一个第二吸收信号，从具有活组织中的 CH 基团吸收峰的波长区检测一个第三吸收信号。通过光谱分析结果的多变量分析确定葡萄糖浓度。

权 利 要 求 书

1、一种使用近红外光谱测定目标中葡萄糖浓度的方法，所说的方法包括以下步骤：

将近红外辐射投射到所说目标上；

接收从所说目标散射的所产生的辐射；

对所产生的辐射进行光谱分析，从具有来自葡萄糖分子的OH基团吸收峰的第一波长区检测至少一个第一吸收信号，从具有所说目标中NH基团吸收峰的第二波长区检测至少一个第二吸收信号，以及从具有所说目标中CH基团吸收峰的第三波长区检测至少一个第三吸收信号；和

通过对所说光谱分析结果的多变量分析确定所说葡萄糖浓度，其中所说的第一、第二和第三吸收信号被用作解释性变量，并且所说的葡萄糖浓度为判断标准变量。

2、按照权利要求1所述的方法，其特征在于所说的第一波长区是在1550 nm至1650nm的范围内，所说的第二波长区是在1480nm至1550nm的范围内，所说的第三波长区是在1650nm至1880nm的范围内。

3、按照权利要求1所述的方法，其特征在于所说的第一波长区是在1050 nm至1130nm的范围内，所说的第二波长区是在1000nm至1050nm的范围内，所说的第三波长区是1130nm至1300nm的范围内。

4、按照权利要求1所述的方法，其特征在于所说的第一波长区是在1600±40nm的范围内，所说的第二波长区是在1530±20nm的范围内，所说的第三波长区是在选自1685±20nm，1715±20nm，和1740±20nm的范围内。

5、按照权利要求1所述的方法，其特征在于所说的第一吸收信号是在所说的第一波长区中在第一波长的吸收能力，所说的第二吸收信号是在所说的第二波长区中在第二波长的吸收能力，所说的第三吸收信号是在第三波长区中在第三波长的吸收能力，并且其中所说的第一，第二

和第三波长是由下述步骤确定的：

制备多个在一个包括白蛋白，葡萄糖和水的系统中具有不同浓度的待测样品；

测定所说的待测样品的吸收谱；

进行所说的吸收谱的多变量分析，得到表明波长和回归系数之间关系的曲线；

在所说的第一波长区内选出基本上对应于所说回归系数一个峰的一个波长作为所说的第一波长，在所说的第二波长区内选出基本上对应于所说回归系数一个峰的一个波长作为所说的第二波长，在所说的第三波长区内选出基本上对应于所说的回归系数一个峰的一个波长作为所说的第三波长。

6、按照权利要求5所述的方法，其特征在于除了所说的第一，第二和第三吸收信号外，第四和第五吸收信号被用作所说的解释性变量，所说的第四和第五吸收信号分别为在第四和第五波长的吸收能力，并且其中所说的第四和第五波长通过下述步骤确定：

进行关于不同主成分的所说的吸收谱的所说多变量分析，得到多个表示波长和回归系数之间关系的曲线；

在所说的第一和第二波长区之间的边界附近选择基本上对应于所说曲线交会处的一个波长作为所说的第四波长，并在所说的第二和第三波长区之间的边界附近选择基本上对应于所说曲线交会处的一个波长作为第五波长。

7、按照权利要求1所述的方法，其特征在于所说的第一吸收信号是在所说第一波长区内在第一波长的吸收能力，所说的第二吸收信号是在所说的第二波长区内在第二波长的吸收能力，所说的第三吸收信号是在所说的第三波长区内在第三波长的吸收能力，并且其中所说的第一，第二和第三波长由下述步骤确定：

对一受试者进行葡萄糖耐受试验；

在所说的葡萄糖耐受试验期间测定所说的受试者的吸收谱；

进行所说吸收谱的多变量分析，得到表示波长和回归系数之间关系

的曲线；

在所说的第一波长区内选择基本上对应于所说回归系数一个峰的波长作为所说的第一波长，在所说的第二波长区内选择基本上对应于所说回归系数一个峰的波长作为所说的第二波长，在所说的第三波长区内选择基本上对应于所说回归系数一个峰的波长作为所说的第三波长。

8、按照权利要求1所述的方法，其特征在于投射在所说目标上的所说近红外辐射实质上由具有在所说的第一波长区内的中心波长和半宽度的第一近红外辐射，具有在所说第二波长区内的中心波长和半宽度的第二近红外辐射和具有在所说第三波长区内的中心波长和半宽度的第三近红外辐射组成。

9、按照权利要求8所述的方法，其特征在于所说的第一近红外辐射的所说的中心波长和所说的半宽度是由下述步骤确定的：

制备多个在包括白蛋白、葡萄糖和水的系统中具有不同浓度的待测样品；

测定所说的待测样品的吸收谱；

进行所说的吸收谱的多变量分析，得到表示波长和回归系数之间的关系的曲线；

在所说的第一波长区内选择基本上对应于所说回归系数最大值的波长作为所说中心波长，并在所说的第一波长区内选择基本上对应于所说最大值的70%或70%以上的波长区作为所说的半宽度。

10、按照权利要求9所述的方法，其特征在于所说的第一近红外辐射具有在15 60nm至1640nm范围内的所说中心波长，和60nm或60nm以下的所说的半宽度。

11、按照权利要求8所述的方法，其特征在于所说的第一近红外辐射的所说中心波长和所说半宽度是通过下述步骤确定的：

对一受试者进行葡萄糖耐受试验；

在所说的葡萄糖耐受试验期间测定所说受试者的吸收谱；

进行所说的吸收谱的多变量分析，得到表示波长和回归系数之间关系的曲线；

在所说的第一波长区内选择基本上对应于所说回归系数最大值的波长作为所说的中心波长，并在所说的第一波长区内选择基本上对应于所说最大值的70%或70%以上的波长作为半宽度。

说　　明　　书

用近红外光谱测定目标中葡萄糖浓度的方法

本发明涉及用近红外光谱测定目标中葡萄糖浓度的方法，特别涉及非侵入性测定受试者血液中葡萄糖浓度的方法。该方法可用于家庭健康检查，或在医疗机构对病人如糖尿病人进行血糖测定。

近红外光谱已广泛应用于各种技术领域，如农业、食品工业，或石油化学，因为它是一种非破坏性检查，不需要为制备待测样品而进行特别的操作。由于近红外辐射是一种低能量的电磁波，可避免样品辐射损伤的发生。近红外辐射与中红外辐射相比难于被水吸收，因而可以检查呈水溶液状态的样品。此外，近红外辐射高剂量透过活的有机体是有利的。

反过来，在近红外辐射的波长范围内，吸收谱的强度非常弱，如为中红外辐射波长范围内吸收谱的强度的约1/100。此外，使用近红外辐射存在难以分清从活体测到的吸收谱的分配。这些问题阻碍了使用近红外光谱进行葡萄糖浓度的精确定量测定。

美国专利NO.4655225公开了一种非侵入性测定体组织中葡萄糖浓度的分光光度方法。将一束由一个方向性的光源射出的光线照射到一选定的机体部位，然后收集从该机体部位散发的由此产生的辐射。所收集的辐射包括波长为1575nm，1756nm，2100nm和2270±15nm的至少一个带，这是典型的葡萄糖吸收谱，和在1000nm至2700nm参照波长范围内的至少一个带，这是典型的背景组织吸收谱。在参照波长（reference wave length）内葡萄糖的吸收为零或不显著。在将吸收的辐射转化成电信号后，受试者的葡萄糖浓度将由电子计算机根据该电信号计算出来。

另一方面，美国专利NO.5070874公开了一种非侵入性测定病人体内葡萄糖浓度的方法。将约1660nm有限波长范围内的近红外辐射投射到病人身体的一个部位上，然后检测从该部位上散射的所产生的辐射。得到

一个所产生辐射强度作为波长的函数的表达式。将该表达式的二阶导数在约1660nm非常窄的范围内，如在 1640nm和1670nm之间扩展。病人的葡萄糖浓度是在该导数的最大或最小点从所产生辐射的强度来确定的。

此外，当使用近红外光谱测定活组织中的葡萄糖浓度时，活组织中水和除葡萄糖之外的组分的吸收谱存在着与葡萄糖的吸收谱重合的倾向。图12和13显示了水、葡萄糖（粉状）、白蛋白（粉状）和胆固醇（粉状）的吸收谱，分别都是在第一和第二谐波的波长频带范围内测定的。例如，检测包括水、葡萄糖和白蛋白的目标的吸收谱时，期望水和白蛋白的吸收谱在约1580nm附近重叠葡萄糖的吸收谱的一个宽峰，如图12所理解的。为改善定量分析葡萄浓度的精确度，将这些干扰因子的影响考虑进葡萄糖的吸收谱中是很重要的。

因而，仍有余地进一步改进现有技术的测定葡萄糖浓度的方法。

本发明的主要目的是提供一种具有改进精确度的用近红外光谱测定目标中葡萄糖浓度的方法。即将近红外辐射投射到目标上，接收从该目标散发的所产生的辐射。将所产生的辐射进行光谱分析，从具有来自葡萄分子的ＯＨ基团吸收峰的第一波长区检测至少一个第一吸收信号，从目标中具有ＮＨ吸收峰的第二波长区检测至少一个第二吸收信号，从目标中具有ＣＨ基团吸收峰的第三波长区检测至少一个第三吸收信号。通过光谱分析结果的多变量分析确定葡萄糖浓度，其中，该第一，第二和第三吸收信号被用作解释性变量，葡萄糖浓度为判断标准变量。

当光谱分析是在第一谐波频带（harmonic tone）区上进行时，优选第一波长区是在1550nm至1650nm范围内，第二波长区是在1480nm至1550nm的范围内，和第三波长区是在1650nm至1880nm的范围内。

当光谱分析是在第二谐波频带区上进行时，优选第一波长区是在1050nm至1130nm的范围内，第二波长区是在1000nm至1050nm的范围内，第三波长区是在1130nm至1300nm的范围内。

也优选第一波长区是在1600±40nm的范围内，第二波长区是在1530±20nm的范围内，第三波长区最好选自1685±20nm，1715±20nm和1740±20nm。

在本发明的一个优选实施方案中，另一吸收信号是一个在第一波长区中的第一波长吸收能力，第二吸收信号是在第二波长区中的第二波长

的吸收能力，第三吸收信号是在第三波长区中的第三波长的吸收能力。该第一，第二和第三波长可通过下列步骤确定。制备在一个包括白蛋白，葡萄糖和水的系统中具有不同浓度的多个待测样品，测定样品的吸收谱。或者对一受试者进行葡萄糖耐受试验，测定在葡萄糖耐受期间受试者的吸收谱。将测到的吸收谱进行多变量分析，得到表示波长和回归系数之间关系的曲线。在这一曲线中，在第一波长区内选择一基本上相应于回归系数的峰的波长作为第一波长。在第二波长区内选择一基本上相应于回归系数的峰的波长作为第二波长。在第三波长区内选择一基本上对应于回归系数的峰的波长作为第三波长。

在本发明的另一个优选实施方案中，投射在目标上的近红外辐射基本上由一个具有在第一波长区内的中心波长和半宽度（half-width）的第一近红外辐射，一个具有在第二波长区内的中心波长和半宽的第二近红外辐射，和一个具有在第三波长区内的中心波长和半宽的第三近红外辐射组成。例如，第一近红外辐射的中心波长和半宽可通过下述步骤确定。制备多个在包括白蛋白、葡萄糖和水的系统中具有不同浓度的待测样品。测量该待测样品的吸收谱。或者对一受试者进行葡萄糖耐受试验，测量在葡萄糖耐受期间受试者的吸收谱。将测到的吸收谱进行多变量分析，得到表示波长和回归系数之间关系的曲线。在这一曲线中，在第一波长区域内选择基本上相应于回归系数最大值的波长作为第一近红外辐射的中心波长，在第一波长区内选择基本上对应于最大值70％或70％以上的波长作为第一近红外辐射的半宽。具体地说，优选该第一近红辐射的中心波长被确定在1560nm至1640nm的范围内，其半宽为60nm或60nm以下。

这些和其它的目的和优点将通过下文结合附图对本发明优选实施方案的描述进行说明。

附图简要说明：

图 1 是显示本发明第一实施例中波长和回归系数之间关系的曲线图。

图 2 是显示本发明第二实施例中波长和回归系数之间关系的曲线图。

图 3 是显示本发明第三实施例中波长和回归系数之间关系的曲线

图。

图 4 是显示本发明第四实施例中使用的用于非侵入性测定受试者血液中葡萄糖浓度的装置的示意图。

图 5 是第四实施例中使用的光纤维束的端视图。

图 6 是第四实施例中测得的吸收谱。

图 7 是显示第四实施例中波长和回归系数之间关系的曲线图。

图 8 是第四实施例中得到的葡萄糖浓度的校准线。

图 9 是本发明第五实施例中使用的用于非侵入性测定受试者血液中葡萄糖浓度的仪器的示意图。

图10是第五实施例中使用的光纤维束的端视图。

图11是图 3 中曲线的局部放大图。

图12是在第一谐波频带区上测定的葡萄糖，白蛋白，胆固醇和水的吸收谱。

图13是在第二谐波频带区上测定的葡萄糖，白蛋白，胆固醇和水的吸收谱。

优选实施方案的详细说明

第一实施例

本发明的第一个实施例提供了一种使用近红外光谱检测小牛血清样品中葡萄糖浓度的方法。

首先制备多个具有不同葡萄糖和白蛋白浓度的小牛血清样品。白蛋白是一种血液中非常普通的蛋白质组分，在测定葡萄糖浓度的光谱分析中被用作干扰因子。这就是将白蛋白包括在小牛血清样品中的原因。将5 ml葡萄糖水溶液和15ml白蛋白水溶液与80ml小牛血清混合，得到每一个小牛血清样品。在这些小牛血清样品中葡萄糖的浓度为30mg/dl，93mg/dl，155mg/dl，280mg/dl，530mg/dl，和1030mg/dl。这些小牛血清样品中白蛋白浓度为2.24g/dl，2.84g/dl，3.44g/dl，4.64g/dl，和5.84g/dl。因而可以制备出30（5×6）种具有不同葡萄糖和白蛋白浓度的小牛血清样品。在本实施例中，使用了从30个不同的小牛血清样品中任选出的15个小牛血清样品。因而，由于在这些小牛血清样品中葡萄糖，白蛋白和水的浓度有变化，通过简单地确定水和葡萄糖之间的关系难以精确地确定葡萄糖的浓度。为精确地确定葡萄糖的浓度也必须考虑

干扰因子白蛋白的影响。

每一个小牛血清样品的光谱测定是通过使用MAGNA850（由“NICOLET”制造）在算术平均值128，分辨率16，一个检测器DTGS KBr，和一个白光源的条件下进行的。在将测得的吸收信号通过使用储存于一个FT-IR存储器中的参照信号被转换成吸收能力，得到光谱数据之后，将光谱数据在1250nm至1850nm之间进行PLS〔部分最小二乘方（Partial Least Squares）〕回归分析，使用多变量分析（multivariate analysis）的市售软件观察到了第一谐波频带（first harmonic tone）的谐波（harmonics）。在这一PLS回归分析中，葡萄浓度为判断标准变量，吸收能力为解释性变量。图1是显示波长和回归系数之间关系的曲线图，是通过对多个主成分进行分析而得到的。使用第七主成分（n＝7）进行PLS回归分析的结果表明，制备校准线的相关系数为0.996，标准误差（SEP）为28.1mg/dl，证实校准线的相关系数为0.992，标准误差（SEP）为38.1mg/dl。可使用主成分分析代替PLS回归分析。

其次，作为葡萄糖浓度校准线的多元回归方程通过下述步骤确定。该多元回归方程用下式表示：

$$Y=a1x1+a2x2+a3x3+a0$$

其中x1，x2和x3是解释性变量，y是判断标准变量，a1，a2和a3是回归系数，a0为常数。该判断标准变量为葡萄糖浓度。解释性变量x1至x3由图1曲线确定。就是在约1590nm处的吸收能力用作解释性变量（x1）。波长1590 nm基本上对应于具有来自葡萄糖分子OH基团吸收峰的第一波长区（1550-1650nm）中观察到的正峰（positive peak）波长，如图1的曲线（n＝7）所示。将在约1525nm处的吸收能力用作解释性变量（x2）。波长1525nm对应于在具有小牛血清样品中的NH基团吸收峰的第二波长区（1480-1550nm）中观察到的负峰（negative peak）附近的波长。将在约1690nm处的吸收能力用作解释性变量（x3）。波长1690nm对应于在具有小牛血清样品中CH基团吸收峰的第三波长区（1650-1850nm）中观察到的负峰附近的波长。

通过使用判断标准变量和这些解释性变量来进行多变量分析，以确定回归系数（ a1-a3）和常数a0，并完成校准线。多变量分析的结果表明，制作校准线的相关系数为0.983，标准误差（SEP）为57.0mg/dl，证实校准线的相关系数为0.981，标准误差（SEP）为60.1mg/dl。

第二实施例

本发明的第二实施例提供了一种使用近红外光谱测定小牛血清样品中葡萄糖浓度的方法。

首先制备多个具有不同葡萄糖和白蛋白浓度的小牛血清样品。将5 ml葡萄糖水溶液和15ml白蛋白水溶液与80ml小牛血清混合，得到每一个小牛血清样品。在这些小牛血清样品中葡萄糖的浓度为35mg/dl，136mg/dl，220mg/dl，412mg/dl，和750mg/dl。在这些小牛血清样品中白蛋白浓度为2.6g/dl，3.0g/dl，3.3g/dl，4.0g/dl，和5.4g/dl。因而，可以制备出25（5×5）个具有不同葡萄糖和白蛋白浓度的小牛血清样品。在本实施例中，使用了从25个不同的小牛血清样品中任选出的13个小牛血清样品。

以与第一实施例相同的步骤对每一个小牛血清样品进行光谱测定，不同的是将探测器用液氮冷却。在将测得的吸收信号用储存在FT-IR存储器中的参照信号转换成吸收能力，得到光谱数据之后，在900nm至1350nm之间进行光谱数据的PLS回归分析，其中，通过使用多变量分析的市售软件观察到第二谐波频带的谐波。将用17点的移动平均方法（moving average method）平坦化的（smoothed）吸收谱进行PLS回归分析。在该PLS回归分析中，葡萄糖浓度为判断标准变量，吸收能力为解释性变量。图 2 是显示波长和回归系数之间关系的曲线图，这是通过对多个主成分进行分析而得到的。使用第七主成分（n＝7）进行PLS回归分析的结果表明，制作校准线的相关系数为0.981，标准误差（SEP）为53.1mg/dl，证实校准线的相关系数为0.959，标准误差（SEP）为77.2mg/dl。

在本实施例中，葡萄糖浓度的测定是通过使用具有来自白蛋白分子ＮＨ基团的负峰1020nm附近的吸收能力、具有来自葡萄糖分子ＯＨ基团正峰的1070nm附近的吸收能力、以及具有来自白蛋白分子ＣＨ基团负峰的1150nm附近的吸收能力来进行的，如图 2 所示。

第三实施例

本发明的第三实施例提供了一种使用近红外光谱检测小牛血清样品中葡萄糖浓度的方法。

首先制备多个具有不同浓度的葡萄糖，白蛋白，胆固醇，中性脂肪和水的小牛血清样品。这些小牛血清中葡萄糖的浓度为35mg/dl，85mg/dl，140mg/dl，220mg/dl，270mg/dl，415mg/dl，510mg/dl，800mg/dl，985mg/dl，1500mg/dl。这些小牛血清样品中白蛋白的浓度为2.2g/dl，2.3g/dl，2.4g/dl，2.5g/dl，2.8g/dl，3.4g/dl，4.5g/dl和5.4g/dl。这些小牛血清样品中胆固醇的浓度为55mg/dl，63mg/dl，70mg/dl，75mg/dl，83mg/dl，100mg/dl，135mg/dl，205mg/dl 和350mg/dl。这些小牛血清样品中中性脂肪的浓度为10mg/dl，15mg/dl，20mg/dl，70mg/dl，133mg/dl，250mg/dl和480mg/dl。在本实施例中，使用了从大量的这些浓度的组合中任选的45个小牛血清样品。

用与第一实施例相同的步骤对每一个小牛血清样品进行光谱测定。当将测得的吸收信号转换成吸收能力，得到光谱数据之后， 对这些光谱数据在1480nm至1850nm波长范围内进行PLS回归分析，其中，通过使用市售的多变量分析软件观察到了第一谐波频带的谐波。在这一PLS回归分析中，葡萄糖浓度为判断标准变量，吸收能力被用作解释性变量。图 3 是显示波长和回归系数之间关系的曲线图，是对多个主成分进行分析而得到的。使用第七主成分（n＝7）进行PLS回归分析的结果表明，制作校准线的相关系数为0.992，标准误差（SEP）为48.7mg/dl，证实校准线的相关系数为0.991，标准误差（SEP）为51.1mg/dl。可使用主成分分析代替PLS回归分析。

其次，作为葡萄糖浓度校准线的多元回归方程是用下述步骤确定的。该多元回归方程由下式表示：

$$Y=a1x1+a2x2+a3x3+a4x4+a5x5+a6x6+a7x7+a0$$

其中x1，x2，x3，x4，x5，x6，x7是解释性变量，y 为判断标准变量，a1，a2，a3，a4，a5，a6和a7是回归系数，a0为常数。该标准变量为葡萄糖浓度。解释性变量x1至x7是由图 3 曲线确定的。即在1580nm处的吸收能力被用作解释性变量（x1）。波长1580nm基本上对应于在具有

来自葡萄糖分子OH基团吸收峰的第一波长区（1550-1650nm）中观察到的正峰波长，如图3的曲线（n＝7）所示。在约1520nm处的吸收能力被用作解释性变量（x2）。波长1520nm基本上对应于在具有来自小牛血清样品中的NH基团吸收峰的第二波长区（1480-1550nm）中观察到的负峰波长。在约1685nm，1715nm和1740nm处的吸收能力被分别用作解释性变量（x3，x4，x5）。这些波长基本上对应于在具有来自小牛血清样品中的CH基团吸收峰的第三波长区（1650-1880nm）中观察到的负峰和正峰。在约1540nm处的吸收能力被用作解释性变量（x6）。波长1540nm基本上对应于第一和第二波长区之间边界附近的图3中曲线交会处的波长。在约1645nm处的吸收能力被用作解释性变量（x7）。波长1645nm基本上对应于在第二和第三波长区之间边界附近的图3中曲线交会处的波长。

用判断标准变量和这些解释性变量进行多变量分析，以确定回归系数（a1-a7）和常数a0，并完成校准线。多变量分析的结果表明制作校准线的相关系数为0.989，标准误差（SEP）为55.6mg/dl，证实校准线的相关系数为0.988，标准误差（SEP）为57.8mg/dl。

在多变量分析之前，最好进行一次预处理，从吸收能力中减去基本上相应于图3曲线交会处的波长值。或者最好进行一次将吸收能力除以交会处附近的波长值的预处理。

第四实施例

图4为非侵入性测定受试者血液中葡萄糖浓度的仪器的示意图。该仪器包括一个作为光源的卤灯1，用于将卤灯发出的近红外辐射引向受试者身体部位9的第一光纤维10，用于接收从该身体部位散发的所产生辐射的第二光纤维20，由第一和第二光纤维形成的光纤维束4，一个作为所产生辐射分光镜的平视野型衍射光栅元件5，一个作为吸收信号探测器的阵列型光二极管6，和一个包括一个用于根据阵列型光二极管的输出确定受试者葡萄糖浓度的微型计算机的操作单元8。在操作单元8中，在吸收信号被转化成吸收能力之后，受试者的葡萄糖浓度通过使用预定的校准线来计算。图4中，数字2代表反射镜。数字3代表设置在卤灯1和第一光纤维10之间的透镜系统。数字60代表设置在衍射光栅元件5和第二光纤维20之间的狭缝。数字7代表A／D转换器。

光纤维束 4 是由多个小束组成的，其中每一个第一光纤维10的投射端被安置在纤维束端面上六角图形的中心，如图 5 中的虚线所示，第二光纤维20的 6 个接收端被安置在六角图形的角上。每一小束的一个接收端20a与 X 一轴向上相邻小束共用。每一小束的两个接受端20b与 Y 一轴向上相邻小束共用。

在每一小束中，在第一光纤维10的投射端的中心和相邻的第二光纤维20的接收端中心的距离 L 为0.5mm。最好将距离 L 确定在0.1mm至 2 mm的范围内，更优选在0.2mm到 1 mm的范围内。该光纤维束 4 被设计成选择性地获得来自受试者皮肤的真皮层的光谱信息。在本实施例中，每一个第一和第二光纤维（10，20）的直径为200 μ m。光纤维束 4 的端面通常压在受试者前臂的皮肤表面。最好使用一个压力计和一个固定装置，以使光纤维束 4 以所需要的压力压在皮肤表面上。

本发明的第四实施例提供了一种使用图 4 中的仪器测定受试者血液中葡萄糖浓度的方法。按照下述步骤，对一个30岁的健康男性受试者进行一个实验。将受试者保持休息状态30分钟，然后使受试者吞咽一个部分水解的淀粉药片。该药片的量相当于约75g葡萄糖。使用测血液型的简化的血糖测量仪，从受试者保持休息状态开始的90分钟时间内每隔10分钟对受试者进行一次血葡萄糖浓度的侵入性测定。对受试者从指尖取血。在每一次葡萄糖浓度侵入性测定后 5 分钟时，用图 4 所示的仪器重复 4 次对受试者进行吸收谱的非侵入性测定。测得的受试者的吸收谱的曲线示于图 6 。在本实施例中。所采用的在侵入性和非侵入性测定之间 5 分钟的时间间隔是考虑到指尖血中的葡萄糖浓度和前臂表面皮肤附近的葡萄糖浓度达到一致时所需要的时间间隔。在侵入性测定期间受试者的血葡萄糖浓度在89至134mg/dl的范围内变化。

然后在1350nm至1850nm的波长区内进行PLS回归分析，其中通过使用交叉证实方法（Cross Validation method）观察到了第一谐波频带的谐波。在这一PLS回归分析中，葡萄糖浓度为判断标准变量，吸收能力被用作解释性变量。图 7 是显示波长和回归系数之间的关系的曲线图，是通过关于一个七主成分（n＝7）分析得到的。PLS回归分析的结果表明，制作校准线的相关系数为0.993，标准误差（SEP）为1.9mg/dl，证实校准线的相关系数为0.988，标准误差（SEP）为2.6mg/dl。

作为血液中葡萄糖浓度校准线的多元回归方程是通过下述步骤确定的。该多元回归方程由下或表示：

$$Y=a1x1+a2x2+a3x3+a0$$

其中x1，x2和x3是解释性变量，y是判断标准变量，a1，a2和a3是回归系数，a0为常数。判断标准变量为葡萄糖浓度。解释性变量x1至x3是由图7的曲线确定的。即在约1640nm处的吸收能力用作解释性变量（x1）。波长1640nm基本上对应于具有来自葡萄糖分子OH基团吸收峰的第一波长区（1600±40nm）中观察到的正峰波长，如图7的曲线所示。在约1550nm处的吸收能力被用作解释性变量（x2）。波长1550nm基本上对应于在具有受试者活组织中的NH基团吸收峰的第二波长区（1530±20nm）中观察到的负峰波长。在约1690nm处的吸收能力被用作解释性变量（x3）。波长1690nm基本上对应于在具有活组织的CH基团的吸收峰的第三波长区（1685±20nm）中观察到的负峰的波长。如果需要，最好将受试者的体温用作一个附加的解释性变量。

使用该判断标准变量和这些解释性变量进行多变量分析，确定回归系数（a1-a3）和常数a0，并完成校准线。多变量分析的结果表明，制作校准线的相关系数为0.957，标准误差（SEP）为4.8mg/dl。证实校准线的相关系数为0.949，标准误差（SEP）为5.3mg/dl。图8显示了通过多变量分析得到的校准线。在图8中也画出了从测得的吸收谱推测的葡萄糖浓度。

第五实施例

图9为非侵入性测定受试者血液中葡萄糖浓度的仪器的示意图。该仪器包括一个作为近红外辐射源的光发射二极管1A，一个近红外辐射的分光镜2A，一个收集近红外辐射的透镜3A，用于将所收集的光导向受试者身体部位的第一光纤维10A，用于接收从该身体部位散发的所产生的辐射的第二光纤维20A，由第一和第二光纤维形成的光纤维束4A，一个作为所产生辐射探测器的光二极管5A，和一个用于从光二极管的输出计算葡萄糖浓度的操作单元（未示出）。由第一光纤维10A的投射端和第二光纤维20A的接收端在光纤维束4A的端面上形成的图

形示于图10。每一个第二和第二光纤维（10A，20A）的直径为500μm。第一光纤维投射端的中心和相邻的第二光维20A接收端的中心之间的距离为500μm。

作为光发射二极管1A，在第一和第二谐波频带可以使用InP系统的光发射二极管，在第三谐波频带可使用GaAs系统或GaAlAs系统的光发射二极管。在本实施例中，使用了具有中心波长1600nm和半宽度160nm的InP系统的光发射二极管。分光镜2A是用圆盘30A和在该圆盘中心周围安置的一组第一、第二和第三干涉滤光片（31A，32A，33A）形成的。圆盘30A可被一个马达6A转动，从第一至第三干涉滤光片中选出所需要的一个。第一干涉滤光片31A被用于提供一种具有中心波长1585nm和半宽度60nm的第一近红外辐射。第二干涉滤光片32A被用于提供一种具有中心波长1530nm和半宽度10nm的第二近红外辐射。第三干涉滤光片33A被用于提供一种具有中心波长1680nm和半宽度10nm的第三近红外辐射。

第一近红外辐射的中心波长和半宽是按照图11的曲线确定的，图11取自第4实施例图3的局部放大图。即中心波长1580nm是基本上对应于回归系数最大值的波长，它是在具有来自葡萄糖分子的OH基团吸收峰的第一波长区1550nm至1650nm中观察到的。60nm的半宽度基本上对应于在第一波长区内具有回归系数最大值的70%或70%以上的波长区。当中心波长和半宽度通过上述步骤确定时，其优点可简化测定葡萄糖浓度的操作而不降低推测葡萄糖浓度的精确度。

为替代上述方法，可按照表示波长和回归系数之间关系的曲线确定第一近红外辐射的中心波长和半宽度，所述曲线是通过对一受试者进行葡萄糖耐受试验，测定葡萄糖耐受试验期间的吸收谱，以及对吸收谱进行多变量分析而得到的。中心波长和半宽并不限于本实施例中所使用的那些数值。最好使用具有在1560nm至1640 nm范围内的中心波长和60nm或60nm以下的半宽度的第一近红外辐射。

在由光二极管5A测到的吸收信号被转换成吸收能力之后，通过使用预先储存在操作单元中的校准线来确定葡萄糖的浓度。最好按照前述实施例中任意一个所述的方法来确定校准线。

在多变量分析之前，最好进行一个预处理，从吸收信号或吸收能力

减去近红外区内的波长值。或者最好进行一个将吸收信号或吸收能力除以波长值的预处理。在本实施例中，最好使用选自1540±10nm或1650±10nm范围内的波长作为波长值。在使用900至1350nm的范围时，其中观察到了第二谐波频带的谐波，最好使用选自1060±10nm或1130±10nm范围内的波长作为波长值。

本申请基于1997年3月25日提交的日本专利申请NO. 9-72150，并要求其优先权，其全部内容引入本文作为参考。

附图标记

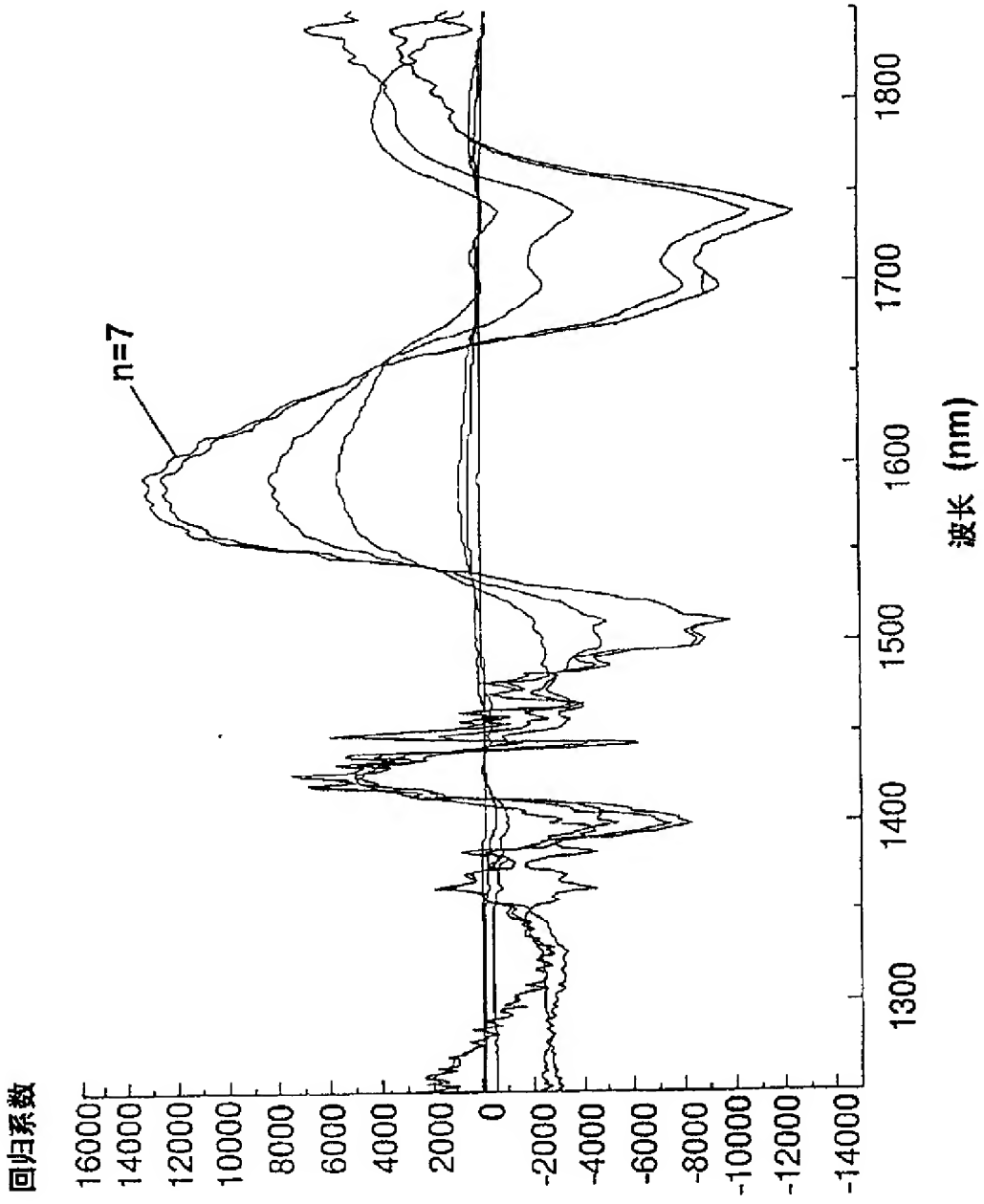
1、卤　灯
2、反射镜
3、透　镜
4、光纤维束
5、平视野型衍射光栅元件
6、阵列型光二极管
7、A / D转换器
8、操作单元
9、身体部位
10、第一光纤维
20、第二光纤维
20a、接收端
20b、接收端
60、狭　缝
1A、光散射二极管
2A、分光镜
3A、透　镜
4A、光纤维束
5A、光二极管
6A、马　达
10A、第一光纤维
20A、第二光纤维

30A、圆　盘

31A、第一干涉滤光片

32A、第二干涉滤光片

33A、第三干涉滤光片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-14000
1300
1400
1500
1600
1700
1800
波长 (nm)
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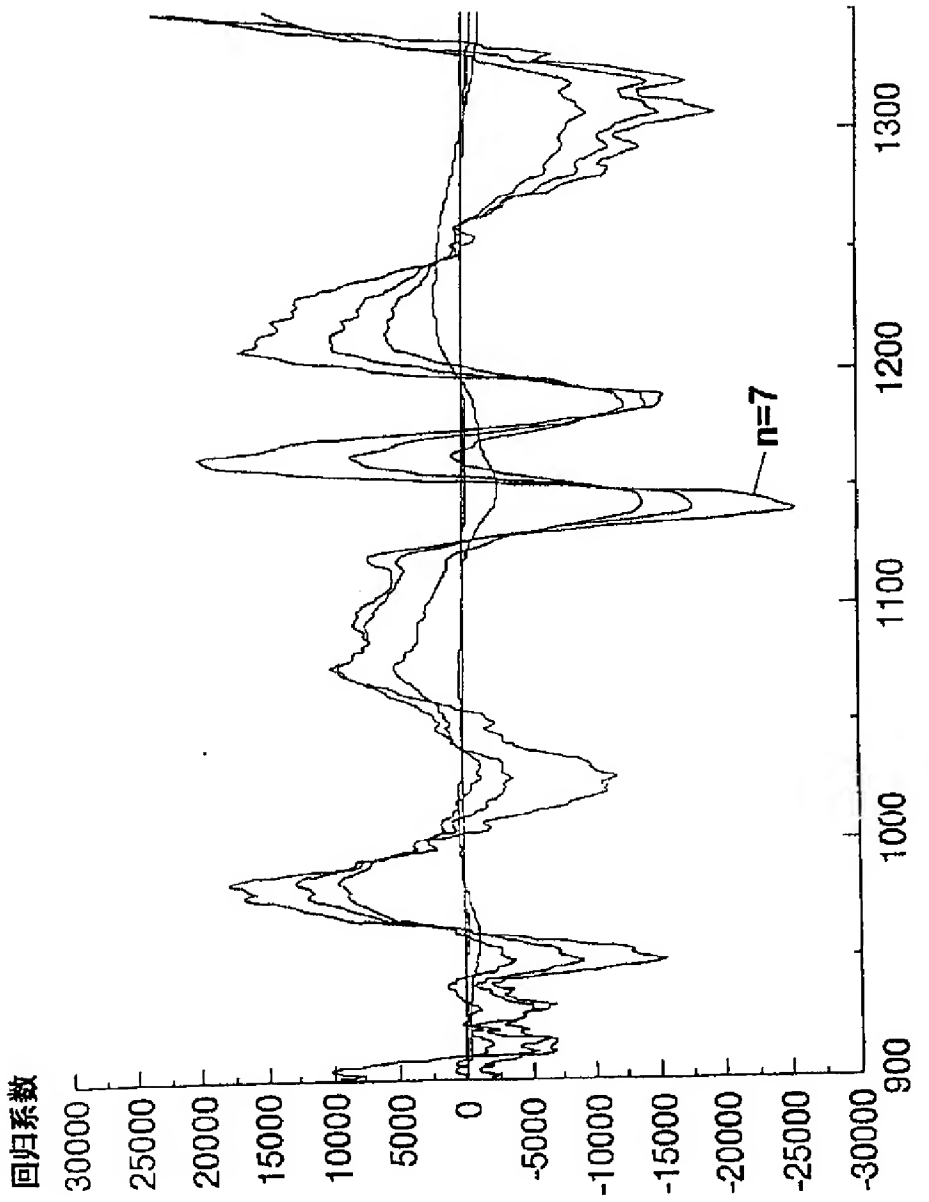
图 1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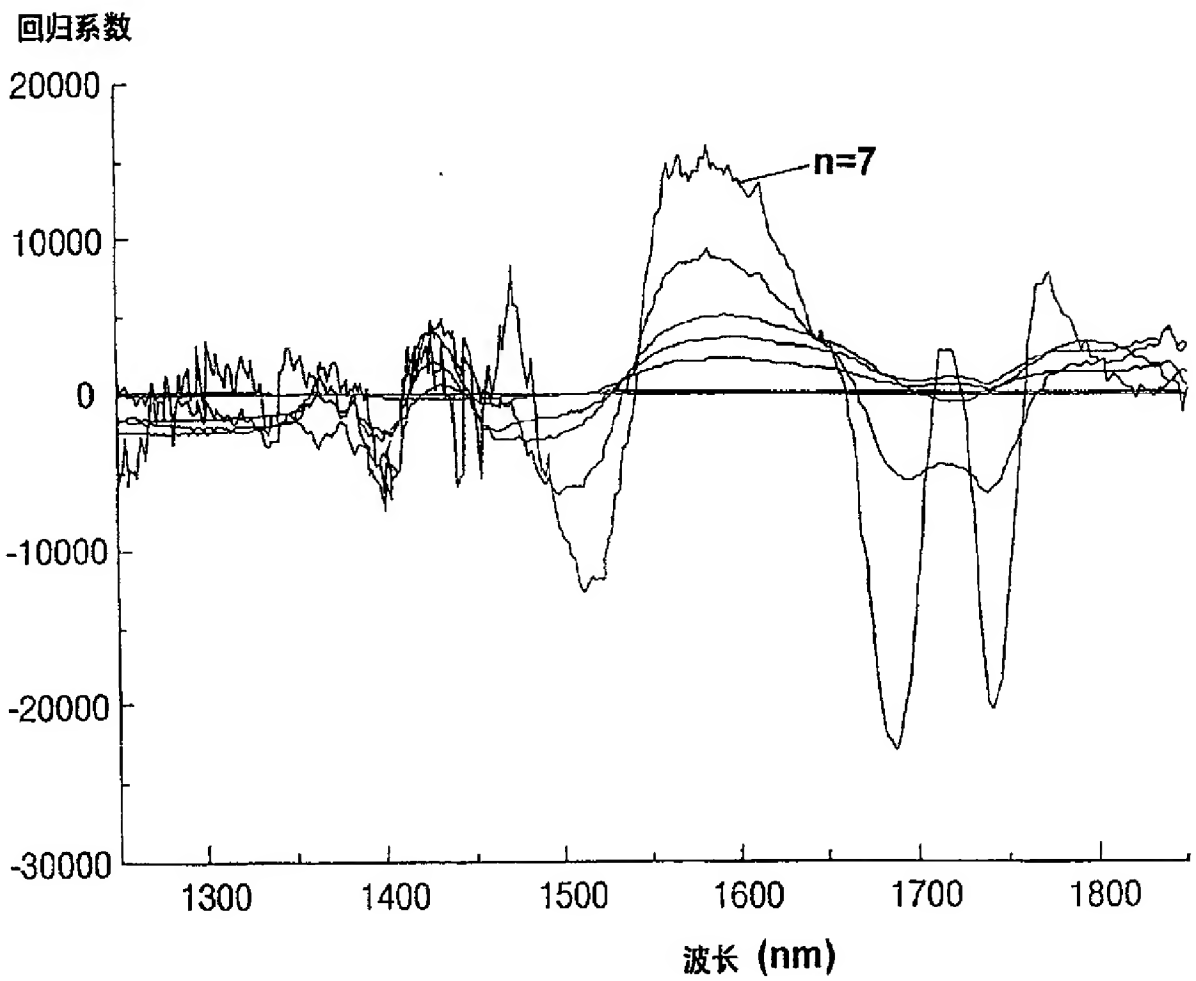
1200
1300
波长 (nm)
n=7

图 2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-20000
-30000
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1400
1500
1600
1700
1800
波长 (nm)

图 3

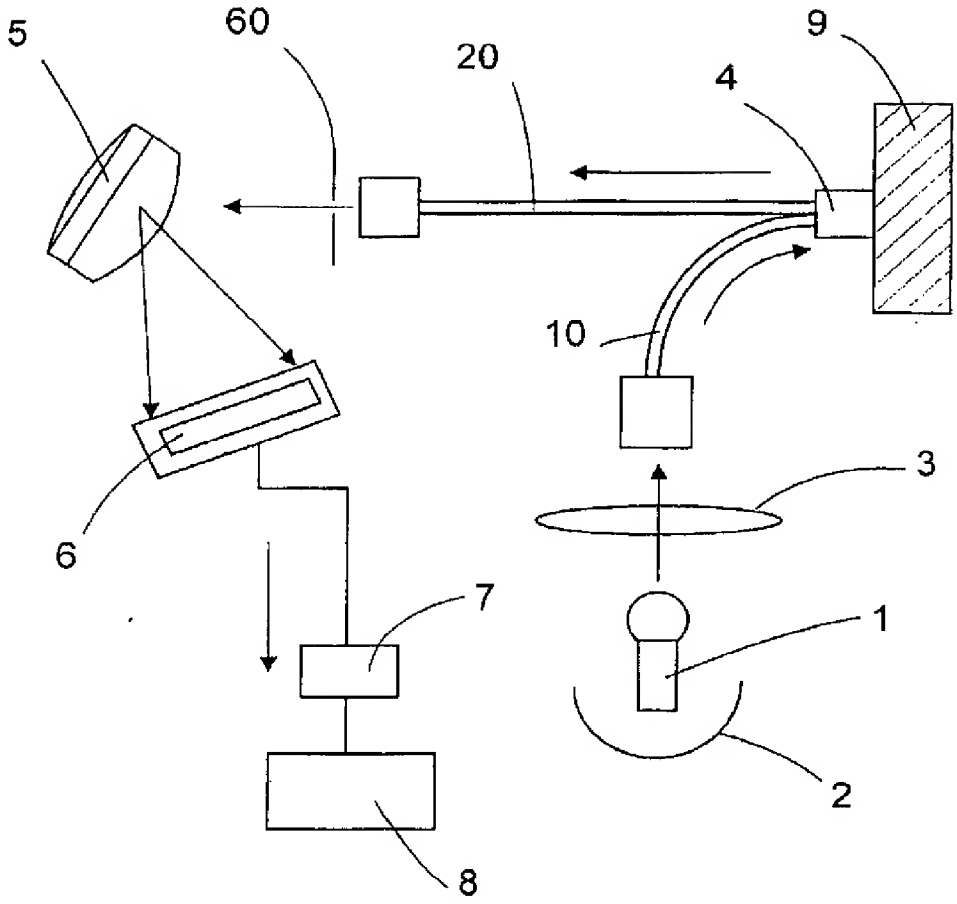


图 4

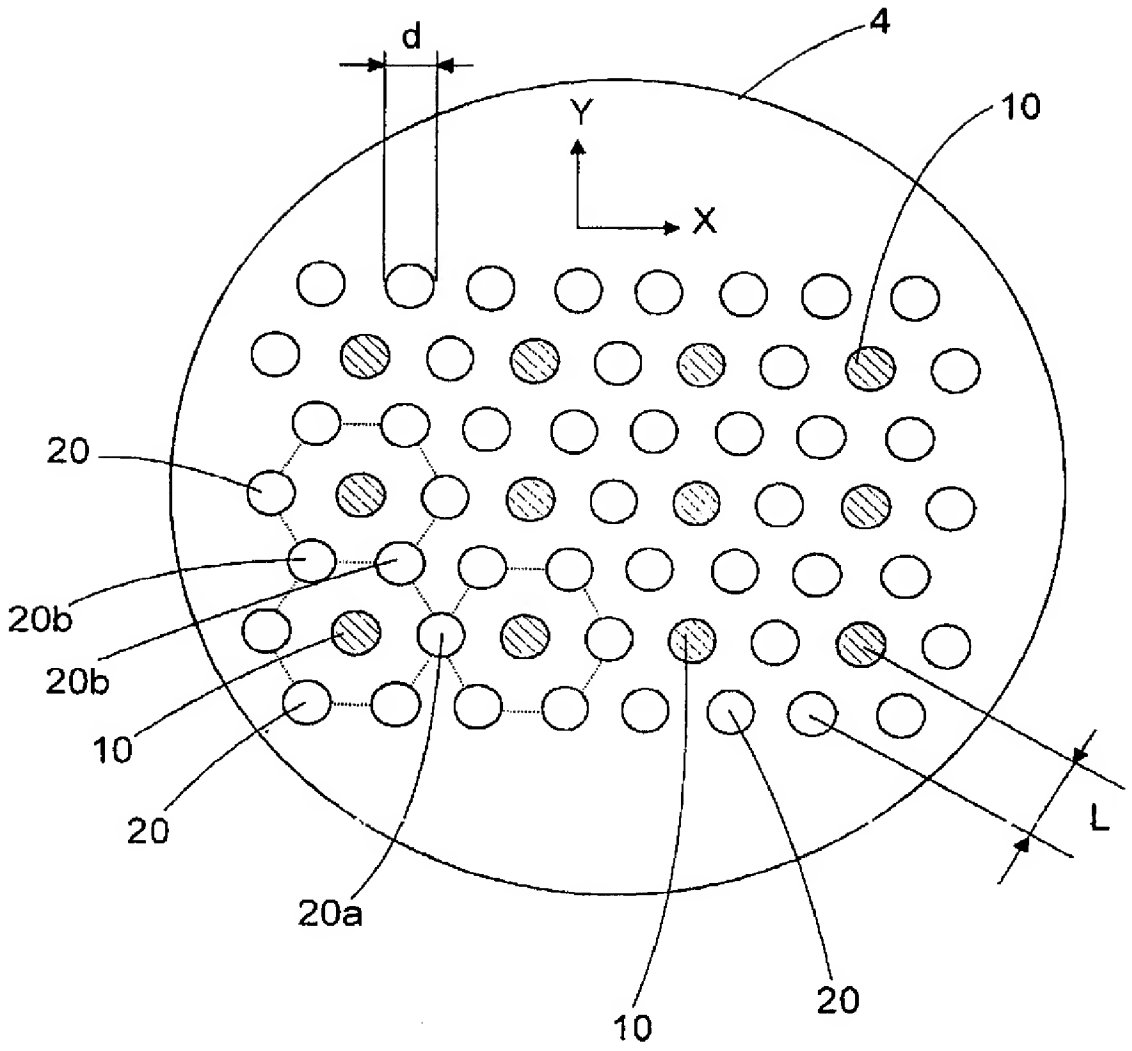


图 5

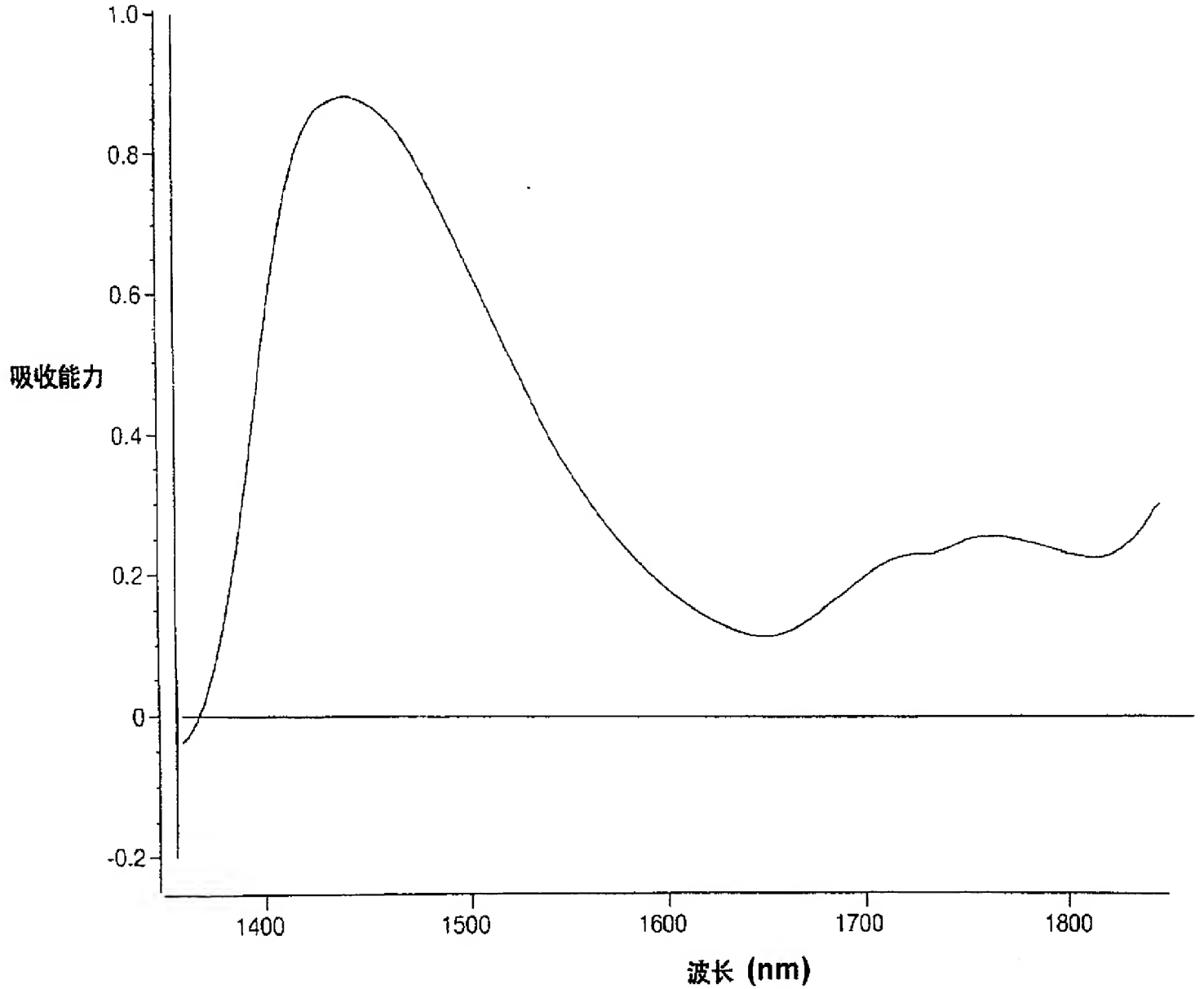


图 6

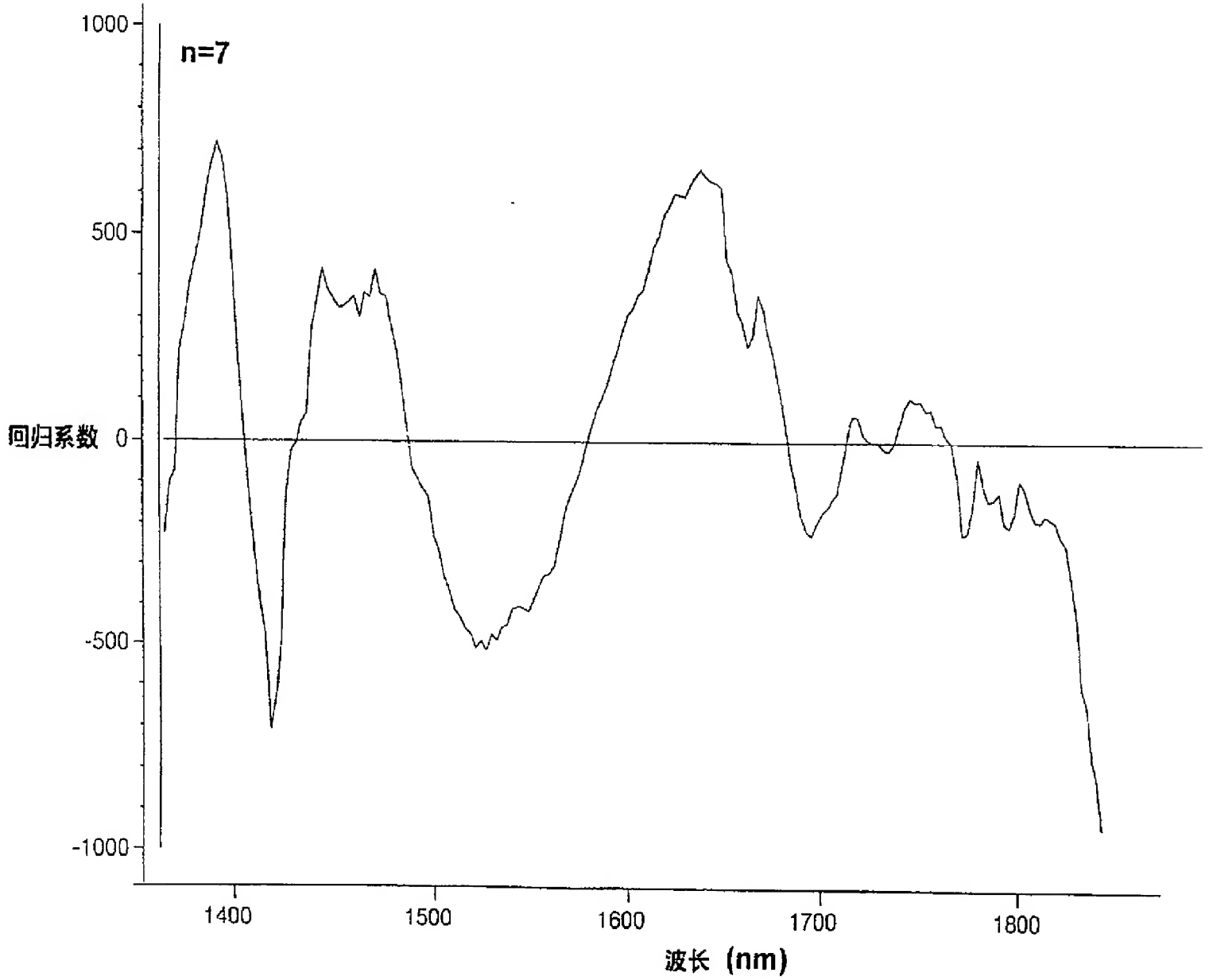
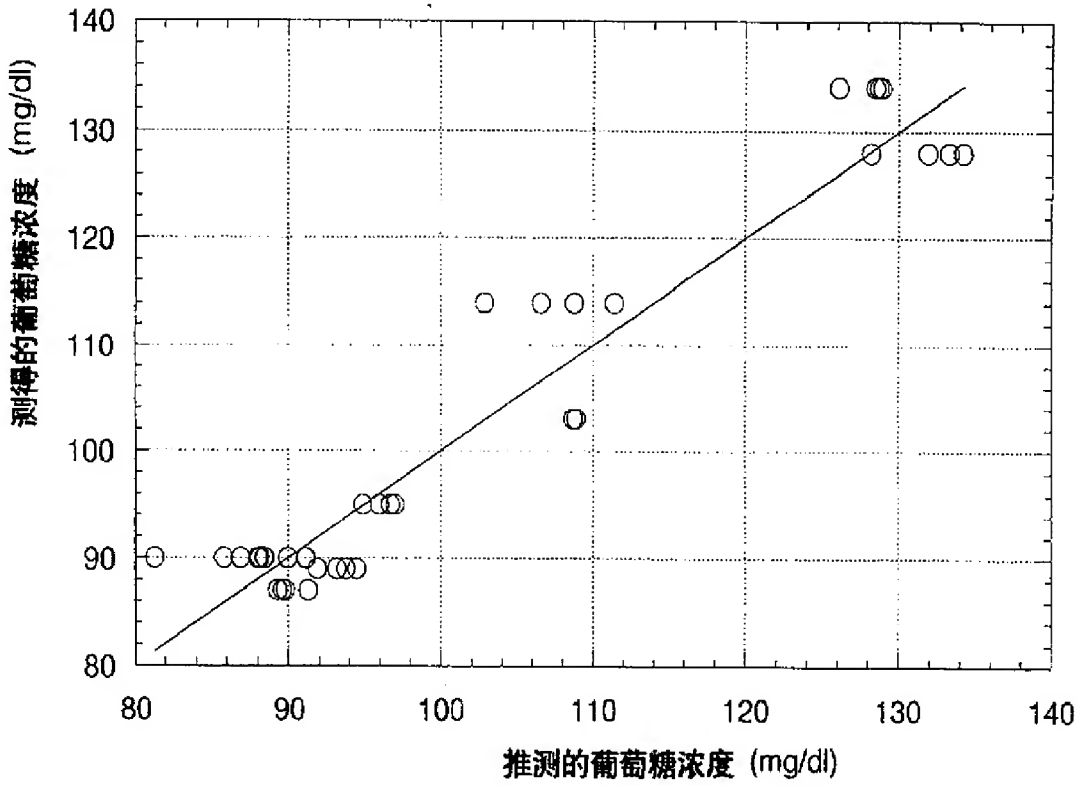


图 7


测得的葡萄糖浓度 (mg/dl)
推测的葡萄糖浓度 (mg/dl)
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130
120
110
100
90
80
80
90
100
110
120
130
140

图 8

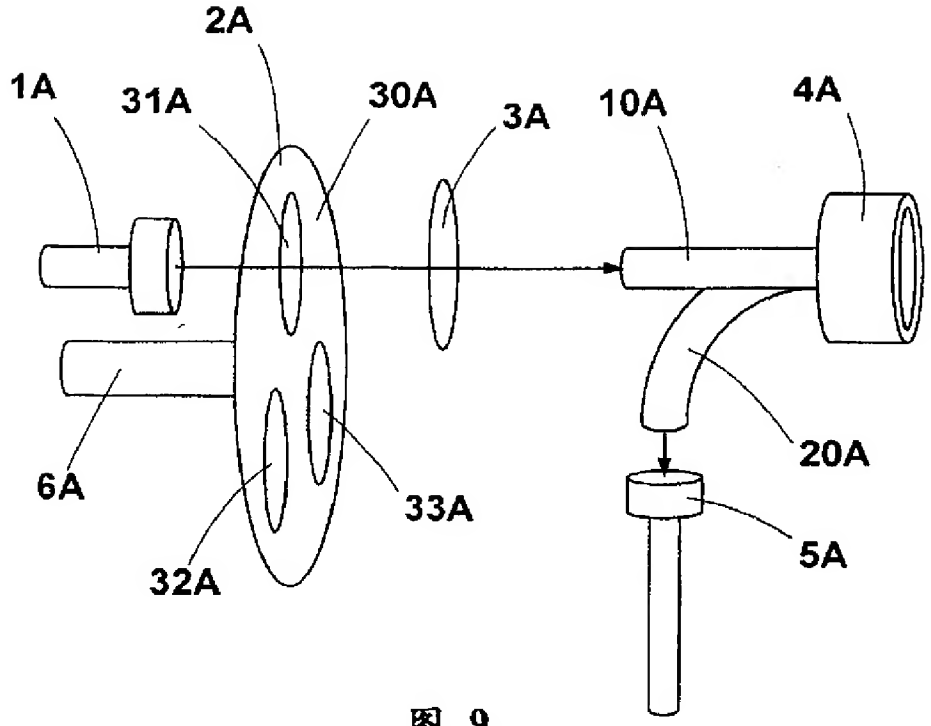


图 9

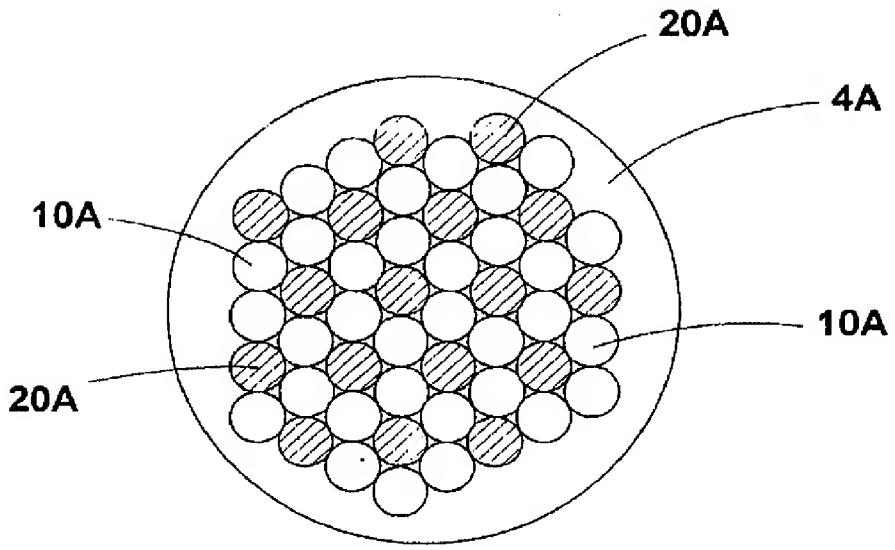
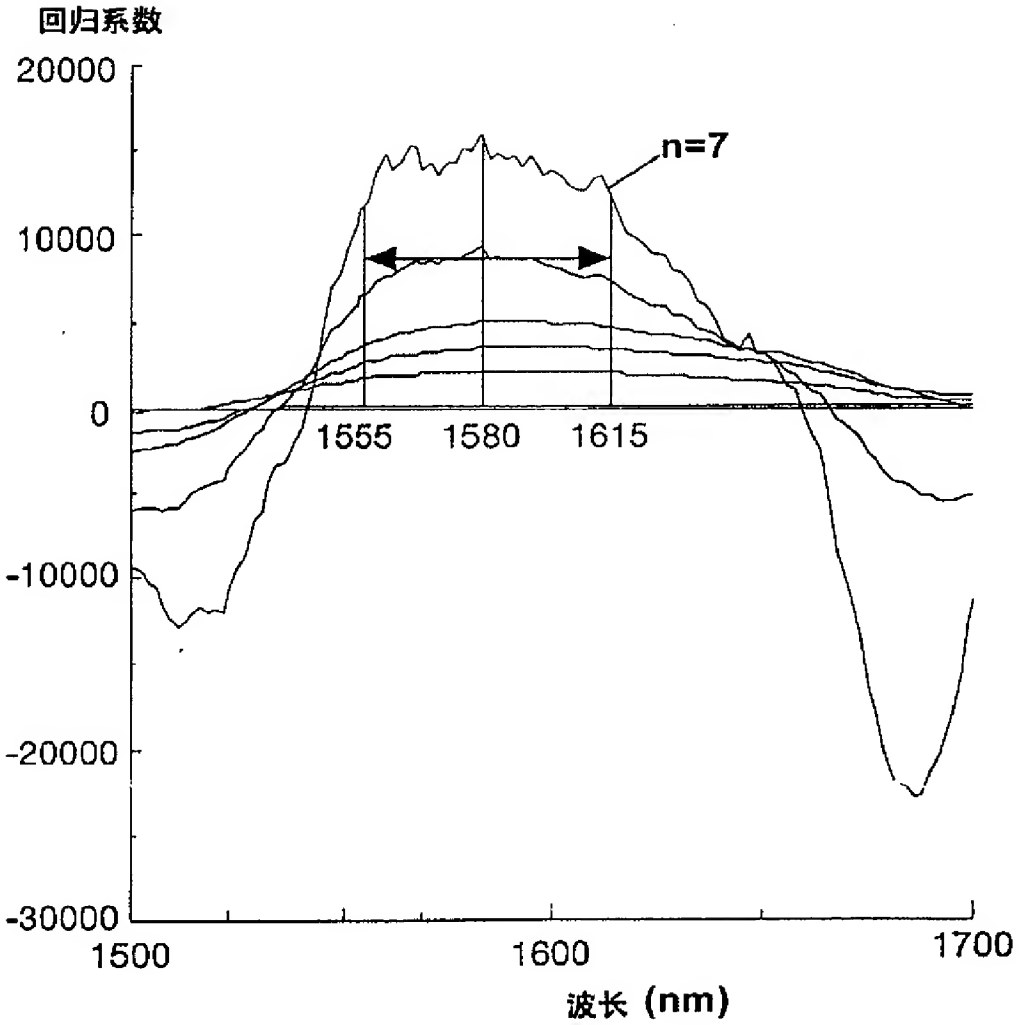


图 10


回归系数
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-30000
n=7
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1615
1500
1600
1700
波长 (nm)

图 11

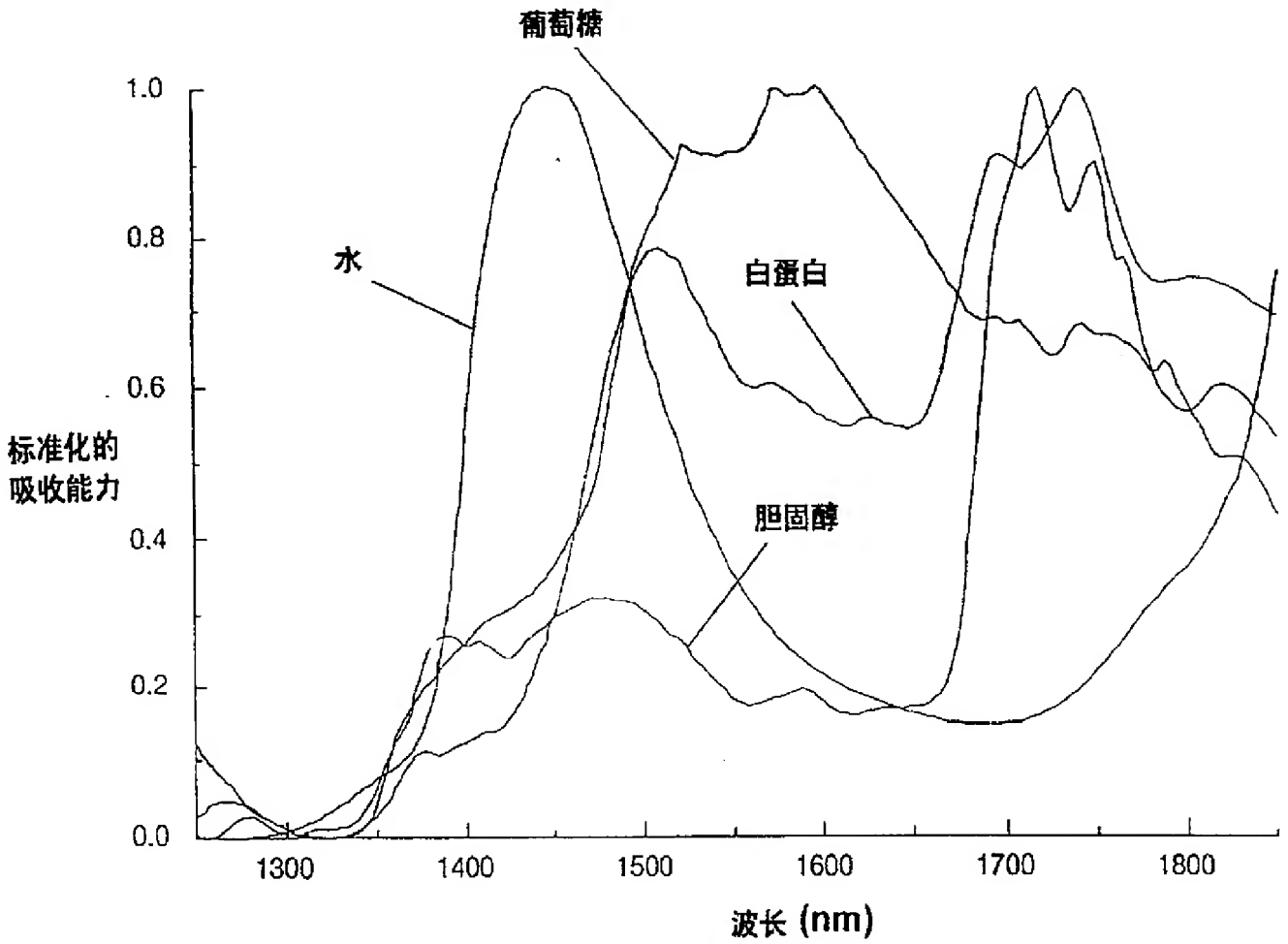
葡萄糖
水
白蛋白
胆固醇
标准化的
吸收能力
1.0
0.8
0.6
0.4
0.2
0.0
1300
1400
1500
1600
1700
1800
波长 (nm)

图 12

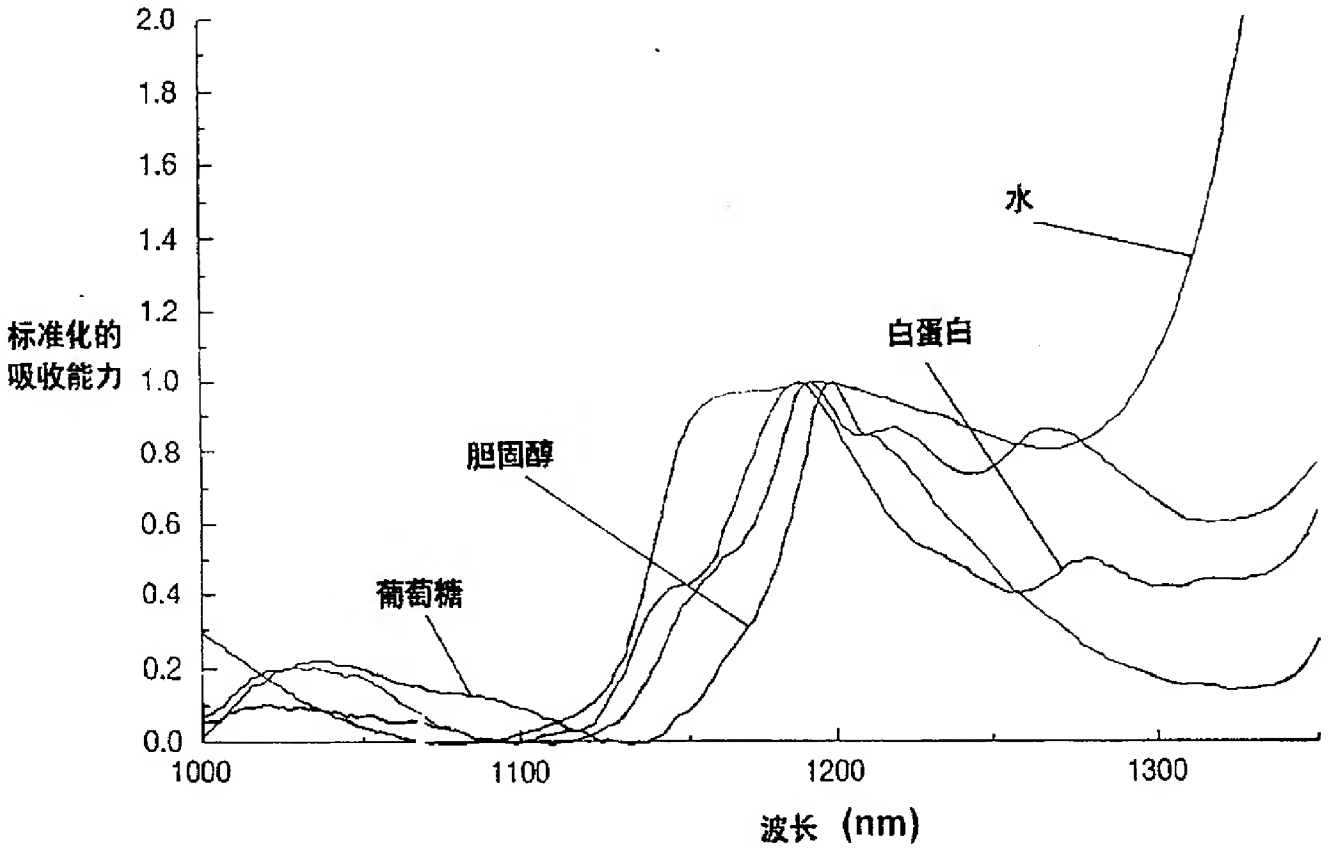
2.0
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
标准化的
吸收能力
水
白蛋白
胆固醇
葡萄糖
1000
1100
1200
1300
波长 (nm)

图 13

THE REQUESTED PATENT IMAGE IS NOT AVAILABLE FROM THE EPO WEBSITE. BELOW IS THE ABSTRACT OF THE REQUESTED PATENT WHICH POSSIBLY LISTS RELATED PATENT DOCUMENTS. IF YOU FEEL THIS MESSAGE IS IN ERROR, PLEASE REATTEMPT DOWNLOADING THE PATENT OR DOUBLE-CHECK THE PATENT IMAGE AVAILABILITY AT http://ep.espacenet.com.

Patent Number:	
Publication date:	1998-12-16
Inventor(s):	KATSUHIKO MARUO (JP); MASAMI OKA (JP)
Applicant(s):	MATSUSHITA ELECTRIC WORKS LTD (JP)
Requested Patent:	CN1201905
Application Number:	CN19981000787 19980324
Priority Number (s):	JP19970072150 19970325
IPC Classification:	G01N21/35; G01N33/66
Equivalents:	CN1112582C, DE69830629D, DE69830629T, EP0869348, A3, B1, KR100268968B, US5957841

Abstract

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CLAIMS
corresponding document: **EP0869348**

Translate this text

1. A method of determining a glucose concentration in a target by using near-infrared spectroscopy, said method comprising the steps of:

projecting near-infrared radiation on said target;
receiving a resulting radiation emitted from said target;
performing a spectrum analysis of the resulting radiation to detect at least one first absorption signal from a first wavelength region having an absorption peak of OH group derived from glucose molecule, at least one second absorption signal from a second wavelength region having an absorption peak of NH group in said target, and at least one third absorption signal from a third wavelength region having an absorption peak of CH group in said target; and
determining said glucose concentration by a multivariate analysis of results of said spectrum analysis, in which said first, second and third absorption signals are used as explanatory variables, and said glucose concentration is a criterion variable.

2. The method as set forth in claim 1, wherein said first wavelength region is in a range of 1550 nm to 1650 nm, said second wavelength region is in a range of 1480 nm to 1550 nm, and said third wavelength region is in a range of 1650 nm to 1880 nm.

3. The method as set forth in claim 1, wherein said first wavelength region is in a range of 1050 nm to 1130 nm, said second wavelength region is in a range of 1000 nm to 1050 nm, and said third wavelength region is in a range of 1130 nm to 1300 nm.

4.The method as set forth in claim 1, wherein said first wavelength region is in a range of 1600 +/- 40 nm, said second wavelength region is in a range of 1530 +/- 20 nm, and said third wavelength region is in a range selected from the group of 1685 +/- 20 nm, 1715 +/- 20 nm, and 1740 +/- 20 nm.

5. The method as set forth in claim 1, wherein said first absorption signal is an absorbency at a first wavelength in said first wavelength region, said second absorption signal is an absorbency at a second wavelength in said second wavelength region, and said third absorption signal is an absorbency at a third wavelength in said third wavelength region, and wherein said first, second and third wavelengths are determined by the steps of:

preparing a plurality of test samples having different concentrations in a system including albumin, glucose, and water measuring absorption spectrums of said test samples;
performing a multivariate analysis of said absorption spectrums to obtain a profile indicative of a relation between wavelength and regression coefficient;
selecting as said first wavelength a wavelength substantially corresponding to a peak of said regression coefficient within said first wavelength region, selecting as said second wavelength a wavelength substantially corresponding to a peak of said regression coefficient within said second wavelength region, and selecting as said third wavelength a wavelength substantially corresponding to a peak of said regression coefficient within said third wavelength region.

6.The method as set forth in claim 5, wherein fourth and fifth absorption signals are used as said explanatory variables in addition to said first, second and third absorption signals, said fourth and fifth absorption signals are absorbencies at fourth and fifth wavelengths, respectively, and wherein said fourth and fifth wavelengths are determined by the steps of:

performing said multivariate analysis of said absorption spectrums with respect to different principal components to obtain a plurality of profiles indicative of relations between wavelength and regression coefficient;
selecting as said fourth wavelength a wavelength substantially corresponding to an intersection of said profiles at the vicinity of a boundary between said first and second wavelength regions, and selecting as said fifth wavelength a wavelength substantially corresponding to an intersection of said profiles at the vicinity of a boundary between said second and third wavelength regions.

7.The method as set forth in claim 1, wherein said first absorption signal is an absorbency at a first wavelength in said first wavelength region, said second absorption signal is an absorbency at a second wavelength in said second wavelength region, and said third absorption signal is an absorbency at a third wavelength in said third wavelength region, and wherein said first, second and third wavelengths are determined by the steps of:

applying a glucose tolerance test to a subject;
measuring absorption spectrums of said subject during said glucose tolerance test;
performing a multivariate analysis of said absorption spectrums to obtain a profile indicative of a relation between wavelength and regression coefficient;
selecting as said first wavelength a wavelength substantially corresponding to a peak of said regression coefficient within said first wavelength region, selecting as said second wavelength a wavelength substantially corresponding to a peak of said regression coefficient within said second wavelength region, and selecting as said third wavelength a wavelength substantially corresponding to a peak of said regression coefficient within said third wavelength region.

8.The method as set forth in claim 1, wherein said near-infrared radiation projected on said target essentially consists of a first near-infrared radiation having a center wavelength and a half-width within said first wavelength region, a second near-infrared radiation having a center wavelength and

a half-width within said second wavelength region, and a third near-infrared radiation having a center wavelength and a half-width within said third wavelength region.

9.The method as set forth in claim 8, wherein said center wavelength and said half-width of said first near-infrared radiation are determined by the steps of:

preparing a plurality of test samples having different concentrations in a system including albumin, glucose, and water measuring absorption spectrums of said test samples;
performing a multivariate analysis of said absorption spectrums to obtain a profile indicative of a relation between wavelength and regression coefficient;
selecting as said center wavelength a wavelength substantially corresponding to a maximum value of said regression coefficient within said first wavelength region, and selecting as said half-width a wavelength region substantially corresponding to 70 % or more of said maximum value within said first wavelength region.

10. The method as set forth in claim 9, wherein said first near-infrared radiation has said center wavelength within a range of 1560 nm to 1640 nm, and said half-width of 60 nm or less.

11. The method as set forth in claim 8, wherein said center wavelength and said half-width of said first near-infrared radiation are determined by the steps of :

applying a glucose tolerance test to a subject;
measuring absorption spectrums of said subject during said glucose tolerance test;
performing a multivariate analysis of said absorption spectrums to obtain a profile indicative of a relation between wavelength and regression coefficient;
selecting as said center wavelength a wavelength substantially corresponding to a maximum value of said regression coefficient within said first wavelength region, and selecting as said half-width a wavelength region substantially corresponding to 70 % or more of said maximum value within said first wavelength region.

DESCRIPTION
corresponding document: **EP0869348**
Translate this text

TECHNICAL FIELD

The present invention relates to a method of determining a glucose concentration in a target by using near-infrared spectroscopy, and particularly a method of non-invasive determination of a glucose concentration in the blood of a subject, which can be used to a health examination at home, or a blood sugar measurement for subjects such as a diabetic at medical facilities.

BACKGROUND ART

Near-infrared spectroscopy has been widely used in various technical fields such as agriculture, food industry, or petrochemistry because it is a kind of non-destructive inspection, and does not need a peculiar operation for preparing a sample to be inspected. Since near-infrared radiation is a low energy electromagnetic wave, it is possible to avoid the occurrence of radiation damage of the sample. Near-infrared radiation is difficult to be absorbed by water as compared with intermediate-infrared radiation, therefore, it is possible to inspect a sample in an aqueous solution state. In addition, there is an advantage of a high transmittance of near-infrared radiation into a living body.

On the contrary, an intensity of absorption spectrum within a wavelength range of near-infrared radiation is very weak, e.g., about 1/100 of the intensity of absorption spectrum within a wavelength range of intermediate-infrared radiation. In addition, there is a problem that it is difficult to clarify the assignment of an absorption spectrum detected from the living body by the use of near-infrared radiation. These problems prevent an accurate quantum analysis of the glucose concentration by using the near-infrared spectroscopy.

US Patent No. 4,655,225 discloses a spectrophotometric method for non-invasive determination of glucose concentration in body tissues. A light provided from a directional optical light source is irradiated on a selected body portion, and then a resulting radiation emitted from the body portion is collected. The collected radiation includes at least one band with a wavelength of 1575 nm, 1756 nm, 2100 nm, and 2270 +/- 15 nm, typical of the glucose absorption spectrum, and at least one band with a reference wavelength in the range of 1000 nm to 2700 nm, typical of the absorption spectrum of background tissue. The absorption of glucose is nil or insignificant at the reference wavelength. After the collected radiation is converted into electrical signals, the glucose concentration of the subject is calculated by an electronic computer according to the electrical signals.

On the other hand, US Patent No. 5,070,874 discloses a method of non-invasive determination of the concentration of glucose in a patient. A near-infrared radiation over a limited range of wavelengths about 1660 nm is projected on a portion of the patient's body, and then the resulting radiation emitted from the portion is sensed. An expression for the magnitude of the resulting radiation as a function of wavelength is derived. The second derivative of the expression in a very narrow range at about 1660 nm, e.g., between 1640 nm and 1670 nm, is expanded. The glucose concentration of the patient is determined from the intensity of the resulting radiation at the maximum or minimum point of this derivative.

By the way, when determining the glucose concentration in a living tissue by using near-infrared spectroscopy, there is a tendency that absorption spectrums of water and components in the living tissue except for glucose overlap the absorption spectrums of glucose. FIGS. 12 and 13 show absorption spectrums of water, glucose (powder), albumin (powder), and cholesterol (powder), which are detected over wavelength ranges of first and second harmonic tones, respectively. For example, when an absorption spectrum of a target including water, glucose and albumin, is detected, it is expected that the absorption spectrums of water and albumin overlap a broad beak of the absorption spectrum of glucose at the vicinity of about 1580 nm, as understood from FIG. 12.In order to improve the accuracy of quantitative analysis of the glucose concentration, it is important to consider the influence of disturbance factors into the absorption spectrums of glucose.

Thus, there is room for further improvement in the methods of determining the glucose concentration of the prior art.

A concern of the present invention is to provide a method of determining a glucose concentration in a target with an improved accuracy by using near-infrared spectroscopy. That is, near-infrared radiation is projected on the target, and a resulting radiation emitted from the target is received. A spectrum analysis of the resulting radiation is performed to detect at least one first absorption signal from a first wavelength region having an absorption peak of OH group derived from glucose molecule, at least one second absorption signal from a second wavelength region having an absorption peak of NH group in the target, and at least one third absorption signal from a third wavelength region having an absorption peak of CH group in the target.The glucose concentration is determined by a multivariate analysis of results of the spectrum analysis, in which the first, second and third absorption signals are used as explanatory variables, and the glucose concentration is a criterion variable.

When the spectrum analysis is performed over a first harmonic tone region, it is preferred that the first wavelength region is in a range of 1550 nm to 1650 nm, the second wavelength region is in a

range of 1480 nm to 1550 nm, and the third wavelength region is in a range of 1650 nm to 1880 nm.

When the spectrum analysis is performed over a second harmonic tone region, it is preferred that the first wavelength region is in a range of 1050 nm to 1130 nm, the second wavelength region is in a range of 1000 nm to 1050 nm, and the third wavelength region is in a range of 1130 nm to 1300 nm.

It is also preferred that the first wavelength region is in a range of 1600 +/- 40 nm, the second wavelength region is in a range of 1530 +/- 20 nm, and the third wavelength region is in a range selected from the group of 1685 +/- 20 nm, 1715 +/- 20 nm, and 1740 +/- 20 nm.

In a preferred embodiment of the present invention, the first absorption signal is an absorbency at a first wavelength in the first wavelength region, the second absorption signal is an absorbency at a second wavelength in the second wavelength region, and the third absorption signal is an absorbency at a third wavelength in the third wavelength region. The first, second and third wavelengths can be determined by the following procedure. A plurality of test samples having different concentrations in a system including albumin, glucose, and water, are prepared, and absorption spectrums of the test samples are measured. Alternatively, a glucose tolerance test is applied to a subject, and absorption spectrums of the subject during the glucose tolerance test are measured.A multivariate analysis of the measured absorption spectrums is performed to obtain a profile indicative of a relation between wavelength and regression coefficient. From this profile, a wavelength substantially corresponding to a peak of the regression coefficient within the first wavelength region is selected as the first wavelength. A wavelength substantially corresponding to a peak of the regression coefficient within the second wavelength region is selected as the second wavelength. A wavelength substantially corresponding to a peak of the regression coefficient within the third wavelength region is selected as the third wavelength.

In a further preferred embodiment of the present invention, the near-infrared radiation projected on the target essentially consists of a first near-infrared radiation having a center wavelength and a half-width within the first wavelength region, a second near-infrared radiation having a center wavelength and a half-width within the second wavelength region, and a third near-infrared radiation having a center wavelength and a half-width within the third wavelength region. For example, the center wavelength and the half-width of the first near-infrared radiation can be determined by the following procedure. A plurality of test samples having different concentrations in a system including albumin, glucose, and water, are prepared, and absorption spectrums of the test samples are measured.Alternatively, a glucose tolerance test is applied to a subject, and absorption spectrums of the subject during the glucose tolerance test are measured. A multivariate analysis of the measured absorption spectrums is performed to obtain a profile indicative of a relation between wavelength and regression coefficient. From this profile, a wavelength substantially corresponding to a maximum value of the regression coefficient within the first wavelength region is selected as the center wavelength of the first near-infrared radiation, and a wavelength region substantially corresponding to 70 % or more of the maximum value within the first wavelength region is selected as the half-width of the first near-infrared radiation.In particular, it is preferred that the center wavelength of the first near-infrared radiation is determined within a range of 1560 nm to 1640 nm, and the half-width thereof is 60 nm or less.

These and still other objects and advantages will become apparent from the following description of the preferred embodiments of the invention when taken in conjunction with the attached drawings.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 is profiles showing relations between wavelength and regression coefficient of a first

embodiment of the present invention;
FIG. 2 is profiles showing relations between wavelength and regression coefficient of a second embodiment of the present invention;
FIG. 3 is profiles showing relations between wavelength and regression coefficient of a third embodiment of the present invention
FIG. 4 is a schematic diagram of a device for non-invasive determination of a glucose concentration in the blood of a subject used in a fourth embodiment of the present invention;
FIG. 5 is an end view of an optical fiber bundle used in the fourth embodiment;
FIG. 6 is an absorption spectrum detected in the fourth embodiment;
FIG. 7 is a profile showing a relation between wavelength and regression coefficient of the fourth embodiment;
FIG. 8 is a calibration line of the glucose concentration obtained in the fourth embodiment;
FIG. 9 is a schematic diagram of a device for non-invasive determination of a glucose concentration in the blood of a subject used in a fifth embodiment of the present invention;
FIG. 10 is an end view of an optical fiber bundle used in the fifth embodiment;
FIG. 11 is a partially-enlarged profile of FIG. 3;
FIG. 12 is absorption spectrums of glucose, albumin, cholesterol, and water, detected over a first harmonic tone region; and
FIG. 13 is absorption spectrums of glucose, albumin, cholesterol, and water, detected over a second harmonic tone region.

DETAIL DESCRIPTION OF THE PREFERRED EMBODIMENTS

&lang&First Embodiment&rang&

The first embodiment of the present invention provides a method of determining a glucose concentration in a bovine serum sample by using near-infrared spectroscopy.

First, a plurality of bovine serum samples having different concentrations of glucose and albumin are prepared. Albumin is a quite ordinary protein component in the blood, and will work as a disturbance factor in a spectrum analysis for determining the glucose concentration. This is the reason why albumin is included in the bovine serum samples. 5 ml of a glucose aqueous solution and 15 ml of an albumin aqueous solution are mixed with 80 ml of a bovine serum to obtain each of the bovine serum samples. The glucose concentrations in the bovine serum samples are 30 mg/dl, 93 mg/dl, 155 mg/dl, 280 mg/dl, 530 mg/dl, and 1030 mg/dl. The albumin concentrations in the bovine serum samples are 2.24 g/dl, 2.84 g/dl, 3.44 g/dl, 4.64 g/dl, and 5.84 g/dl. Therefore, it is possible to prepare the bovine serum samples having 30 (5x6) different concentrations of glucose and albumin.In this embodiment, 15 bovine serum samples optionally selected from the 30 different bovine serum samples are used. Thus, since the concentrations of glucose, albumin and water, are changed in these bovine serum samples, it is difficult to accurately determine the glucose concentration by simply considering a relation between water and glucose. The influence of albumin of the disturbance factor must be also considered to accurately determine the glucose concentration.

A spectrum measurement of each of the bovine serum samples is performed by using MAGNA 850 (manufactured by "NICOLET") under conditions of arithmetic mean 128, resolution 16, a detector DTGS KBr, and a white light source. After the measured absorption signals are converted to absorbencies by using a reference signal stored in a memory in an FT-IR to obtain spectrum data, PLS (Partial Least Squares) regression analysis of the spectrum data is performed over 1250 nm to 1850 nm, in which harmonies of a first harmonic tone are observed, by using a marketed software of multivariate analysis. In this PLS regression analysis, the glucose concentration is a criterion

variable, and the absorbencies are explanatory variables. FIG. 1 is profiles showing relations between wavelength and regression coefficient, which are obtained by analyzing with respect to a plurality of principal components.Results of the PLS regression analysis by the use of a seventh principal component (n=7) show that a correlation coefficient at the preparation of a calibration line is 0.996, a standard error (SEP) is 28.1 mg/dl, a correlation coefficient at the validation of the calibration line is 0.992, and a standard error (SEP) is 38.1 mg/dl. In place of the PLS regression analysis, it is possible to use principal component analysis.

Next, a multiple regression equation as a calibration line of the glucose concentration is determined by the procedure explained below. The multiple regression equation is expressed by the following equation:
$Y = a1x1 + a2x2 + a3x3 + a0$
wherein x1, x2 and x3 are explanatory variables, Y is a criterion variable, a1, a2, and a3 are regression coefficients, and a0 is a constant. The criterion variable is the glucose concentration. The explanatory variables x1 to x3 are determined from the profile of FIG. 1. That is, an absorbency at about 1590 nm is used as the explanatory variable (x1). The wavelength of 1590 nm substantially corresponds to a wavelength of a positive peak observed in a first wavelength region (1550 SIMILAR 1650 nm) having an absorption peak derived from OH group of glucose molecule, as shown in the profile (n = 7) of FIG. 1.An absorbency at about 1525 nm is used as the explanatory variable (x2). The wavelength of 1525 nm corresponds to a wavelength at the vicinity of a negative peak observed in a second wavelength region (1480 SIMILAR 1550 nm) having an absorption peak derived from NH group in the bovine serum sample. An absorbency at about 1690 nm is used as the explanatory variable (x3). The wavelength of 1690 nm corresponds to a wavelength at the vicinity of a negative peak observed in a third wavelength region (1650 SIMILAR 1850 nm) having an absorption peak derived from CH group in the bovine serum sample.

A multivariate analysis is performed by using the criterion variable and these explanatory variables to determine the regression coefficients (a1 -a3) and the constant a0 and complete the calibration line. Results of the multivariate analysis show that a correlation coefficient at the preparation of the calibration line is 0.983, a standard error (SEP) is 57.0 mg/dl, a correlation coefficient at the validation of the calibration line is 0.981, and a standard error (SEP) is 60.1 mg/dl.

&lang&Second Embodiment&rang&

The second embodiment of the present invention provides a method of determining a glucose concentration in a bovine serum sample by using near-infrared spectroscopy.

First, a plurality of bovine serum samples having different concentrations of glucose and albumin are prepared. 5 ml of a glucose aqueous solution and 15 ml of an albumin aqueous solution are mixed with 80 ml of a bovine serum to obtain each of the bovine serum samples. . The glucose concentrations in the bovine serum samples are 35 mg/dl, 136 mg/dl, 220 mg/dl, 412 mg/dl, and 750 mg/dl. The albumin concentrations in the bovine serum samples are 2.6 g/dl, 3.0 g/dl, 3.3 g/dl, 4.0 g/dl, and 5.4 g/dl. Therefore, it is possible to prepare the bovine serum samples having 25 (5x5) different concentrations of glucose and albumin. In this embodiment, 13 bovine serum samples optionally selected from the 25 different bovine serum samples are used.

A spectrum measurement of each of the bovine serum samples is performed by the same procedure as the first embodiment except for the detector is cooled by liquid nitrogen. After the measured absorption signals are converted to absorbencies by using a reference signal stored in a memory in an FT-IR to obtain spectrum data, PLS regression analysis of the spectrum data is performed over 900 nm to 1350 nm, in which harmonics of a second harmonic tone are observed, by using a marketed software of multivariate analysis. The PLS regression analysis is performed to absorption spectrums smoothed by a moving average method by 17 points. In this PLS regression analysis, the glucose

concentration is a criterion variable, and the absorbencies are explanatory variables.FIG. 2 is profiles showing relations between wavelength and regression coefficient, which are obtained by analyzing with respect to a plurality of principal components. Results of the PLS regression analysis by the use of a seventh principal component (n=7) show that a correlation coefficient at the preparation of a calibration line is 0.981, a standard error (SEP) is 53.1 mg/dl, a correlation coefficient at the validation of the calibration line is 0.959, and a standard error (SEP) is 77.2 mg/dl.

In this embodiment, the determination of the glucose concentration is performed by using an absorbency at the vicinity of 1020 nm having a negative peak derived from NH group of albumin molecule, absorbency at the vicinity of 1070 nm having a positive peak derived from OH group of glucose molecule, and an absorbency at the vicinity of 1150 nm having a negative peak derived from CH group of albumin molecule, as shown in FIG. 2.

&lang&Third Embodiment&rang&

The third embodiment of the present invention provides a method of determining a glucose concentration in a bovine serum sample by using near-infrared spectroscopy.

First, a plurality of bovine serum samples having different concentrations of glucose, albumin, cholesterol, neutral fat, and water, are prepared. The glucose concentrations in the bovine serum samples are 35 mg/dl, 85 mg/dl, 140 mg/dl, 220 mg/dl, 270 mg/dl, 415 mg/dl, 510 mg/dl, 800 mg/dl, 985 mg/dl, 1500 mg/dl. The albumin concentrations in the bovine serum samples are 2.2 g/dl, 2.3 g/dl, 2.4 g/dl, 2.5 g/dl, 2.8 g/dl, 3.4 g/dl, 4.5 g/dl, and 5.4 g/dl. The cholesterol concentrations in the bovine serum samples are 55 mg/dl, 63 mg/dl, 70 mg/dl, 75 mg/dl, 83 mg/dl, 100 mg/dl, 135 mg/dl, 205 mg/dl, and 350 mg/dl. The neutral fat concentrations in the bovine serum samples are 10 mg/dl, 15 mg/dl, 20 mg/dl, 70 mg/dl, 133 mg/dl, 250 mg/dl, and 480 mg/dl. In this embodiment, 45 bovine serum samples optionally selected from a large number of combinations of these concentrations are used.

A spectrum measurement of each of the bovine serum samples is performed by the same procedure as the first embodiment. After the measured absorption signals are converted to absorbencies to obtain spectrum data, PLS regression analysis of the spectrum data is performed over a wavelength range of 1480 nm to 1850 nm, in which harmonics of a first harmonic tone are observed, by using a marketed software of multivariate analysis. In this PLS regression analysis, the glucose concentration is a criterion variable, and the absorbencies are used as explanatory variables. FIG. 3 is profiles showing relations between wavelength and regression coefficient, which are obtained by analyzing with respect to a plurality of principal components.Results of the PLS regression analysis by the use of a seventh principal component (n=7) show that a correlation coefficient at the preparation of a calibration line is 0.992, a standard error (SEP) is 48.7 mg/dl, a correlation coefficient at the validation of the calibration line is 0.991, and a standard error (SEP) is 51.1 mg/dl. In place of the PLS regression analysis, it is possible to use principal component analysis.

Next, a multiple regression equation as a calibration line of the glucose concentration is determined by the procedure explained below. The multiple regression equation is expressed by the following equation:

$$Y = a1x1 + a2x2 + a3x3 + a4x4 + a5x5 + a6x6 + a7x7 + a0$$

wherein x1, x2, x3, x4, x5, x6, and x7 are explanatory variables, Y is a criterion variable, a1, a2, a3, a4, a5, a6 and a7 are regression coefficients, and a0 is a constant. The criterion variable is the glucose concentration. The explanatory variables x1 to x7 are determined from the profiles of FIG. 3. That is, an absorbency at 1580 nm is used as the explanatory variable (x1).The wavelength of 1580 nm substantially corresponds to a wavelength of a positive peak observed in a first wavelength region (1550-1650 nm) having an absorption peak derived from OH group of glucose molecule, as shown in the profile (n=7) of FIG. 3. An absorbency at about 1520 nm is used as the explanatory

variable (x2). The wavelength of 1520 nm substantially corresponds to a wavelength of a negative peak observed in a second wavelength region (1480-1550 nm) having an absorption peak derived from NH group in the bovine serum sample. Absorbencies at about 1685 nm, 1715 nm, and 1740 nm, are used as the explanatory variables (x3, x4, x5), respectively. These wavelengths substantially correspond to negative and positive peaks observed in a third wavelength region (1650-1880 nm) having absorption peaks derived from CH group in the bovine serum sample.An absorbency at about 1540 nm is used as the explanatory variable (x6). The wavelength of 1540 nm substantially corresponds to a wavelength of an intersection of the profiles of FIG. 3 at the vicinity of a boundary between the first and second wavelength regions. An absorbency at about 1645 nm is used as the explanatory variable (x7). The wavelength of 1645 nm substantially corresponds to a wavelength of an intersection of the profiles of FIG. 3 at the vicinity of a boundary between the second and third wavelength regions.

A multivariate analysis is performed by using the criterion variable and these explanatory variables to determine the regression coefficients (a1 -a7) and the constant a0, and complete the calibration line. Results of the multivariate analysis show that a correlation coefficient at the preparation of the calibration line is 0.989, a standard error (SEP) is 55.6 mg/dl, a correlation coefficient at the validation of the calibration line is 0.988, and a standard error (SEP) is 57.8 mg/dl.

Prior to the multivariate analysis, it is preferred to perform a pretreatment of subtracting the value of wavelength substantially corresponding to an intersection of the profiles of FIG. 3 from the absorbencies. Alternatively, it is preferred to perform a pretreatment of dividing the absorbencies by the wavelength value at the vicinity of the intersection.

&lang&Fourth Embodiment&rang&

A schematic diagram of a device of non-invasive determination of a glucose concentration in the blood of a subject is shown in FIG. 4. The device comprises a halogen lamp 1 an a light source, first optical fibers 10 for introducing near-infrared radiation provided from the halogen lamp to a body portion 9 of the subject, second optical fibers 20 for receiving a resulting radiation emitted from the body portion, an optical fiber bundle 4 formed with the first and second optical fibers, a flat-field type diffraction grating unit 5 as a spectroscope of the resulting radiation, an array-type photo diode 6 as a detector of the absorption signals, and an operation unit 8 comprising a microcomputer for determining the glucose concentration of the subject according to outputs of the array-type photo diode.In the operating unit 8, after the absorption signals are converted to absorbencies, the glucose concentration of the subject is calculated by the use of a predetermined calibration line. In FIG. 4, numeral 2 designates a reflection mirror. Numeral 3 designates a lens system disposed between the halogen lamp 1 and the first optical fibers 10. Numeral 60 designates a slit disposed between the diffraction grating unit 5 and the second optical fibers 20. Numeral 7 designates an A/D converter.

The optical fiber bundle 4 is formed with a plurality of sub-bundles, in each of which a projection end of the first optical fiber 10 is disposed on an end surface of the bundle at a center of a hexagonal pattern, as shown by a dotted line in FIG. 5, and six receiving ends of the second optical fibers 20 are disposed at corners of the hexagonal pattern. A receiving end 20a of each of the sub-bundles is common with an adjacent sub-bundle in an X-axis direction. Two receiving ends 20b of each of the sub-bundles are common with an adjacent sub-bundle in a Y-axis direction.

In each of the sub-bundles, a distance L between centers of the projection end of the first optical fiber 10 and an adjacent receiving end of the second optical fiber 20 is 0.5 mm. It is preferred to determine the distance L within a range of 0.1 mm to 2 mm, and more preferably a range of 0.2 mm to 1 mm. This optical fiber bundle 4 is designed to selectively extract spectrum information from a dermis layer of the skin of the subject. In this embodiment, a diameter of each of the first and second optical fibers (10, 20) is 200 mu m. The end surface of the bundle 4 is pressed normally against a

skin surface of the forearm of the subject. It is preferred to use a pressure gauge and a fixture for pressing the bundle 4 against the skin surface by a required pressure.

The fourth embodiment of the present invention provides a method of determining the glucose concentration in the blood of a subject by using the device of FIG. 4.

An experiment is performed to a subject of a healthy male, thirty years of age, according to the procedure explained below. The subject is kept at a rest state for 30 minutes, and then a medicine of partial hydrolysate of starch is ingested by the subject. An amount of the medicine corresponds to about 75 g of glucose. An invasive measurement of the glucose concentration in the blood of the subject is performed every 10 minutes for 90 minutes from the start of keeping the subject at the rest state by using a simplified blood sugar measuring device of a blood-taking type. The blood of the subject is taken from the tip of a finger. A non-invasive measurement of absorption spectrums of the subject is repeated four times by using the device of FIG. 4 at the lapse of 5 minutes from each of the invasive measurements of the glucose concentration.A profile of the measured absorption spectrum of the subject is shown in FIG. 6. In this embodiment, the time lag of 5 minutes between the invasive and non-invasive measurements is adopted to consider a time difference necessary for the correspondence between the glucose concentrations in the blood of the tip of finger and in the vicinity of the skin surface of the forearm. The glucose concentration in the blood of the subject is changed within a range of 89 to 134 mg/dl during the invasive measurements.

Next, PLS regression analysis is performed over a wavelength region of 1350 nm to 1850 nm, in which harmonics of a first harmonic tone are observed, by using a cross validation method. In this PLS regression analysis, the glucose concentration is a criterion variable, and the absorbencies are used as explanatory variables. FIG. 7 is a profile showing a relation between wavelength and regression coefficient, which is obtained by analyzing with respect to a seven principal component (n = 7). Results of the PLS regression analysis show that a correlation coefficient at the preparation of a calibration line is 0.993, a standard error (SEP) is 1.9 mg/dl, a correlation coefficient at the validation of the calibration line is 0.988, and a standard error (SEP) is 2.6 mg/dl.

A multiple regression equation as a calibration line of the glucose concentration in the blood is determined by the procedure explained below. The multiple regression equation is expressed by the following equation:

$$Y = a1x1 + a2x2 + a3x3 + a0$$

wherein x1, x2 and x3 are explanatory variables, Y is a criterion variable, a1, a2, and a3 are regression coefficients, and a0 is a constant. The criterion variable is the glucose concentration. The explanatory variables x1 to x3 are determined from the profile of FIG. 7. That is, an absorbency at about 1640 nm is used as the explanatory variable (x1). The wavelength of 1640 nm substantially corresponds to a wavelength of a positive peak observed in a first wavelength region (1600+/-40 nm) having an absorption peak derived from OH group of glucose molecule, as shown in the profile of FIG. 7.An absorbency at about 1550 nm is used as the explanatory variable (x2). The wavelength of 1550 nm substantially corresponds to a wavelength of a negative peak observed in a second wavelength region (1530+/-20 nm) having an absorption peak derived from NH group in the living tissue of the subject. An absorbency at about 1690 nm is used as the explanatory variable (x3). The wavelength of 1690 nm substantially corresponds to a wavelength of a negative peak observed in a third wavelength region (1685+/-20 nm) having an absorption peak derived from CH group in the living tissue. If necessary, it is preferred to use a body temperature of the subject as an additional explanatory variable.

A multivariate analysis is performed by using the criterion variable and these explanatory variables to determine the regression coefficients (a1-a3) and the constant a0 and complete the calibration line. Results of the multivariate analysis show that a correlation coefficient at the preparation of the calibration line is 0.957, a standard error (SEP) is 4.8 mg/dl, a correlation coefficient at the validation of the calibration line is 0.949, and a standard error (SEP) is 5.3 mg/dl. FIG. 8 shows the calibration line obtained by the multivariate analysis. In FIG. 8, glucose concentration values

predicted from the measured absorption spectrums are also plotted.

&lang&Fifth Embodiment&rang&

A schematic diagram of a device of non-invasive determination of a glucose concentration in the blood of a subject is shown in FIG. 9. The device comprises a light-emitting diode 1A as a near-infrared radiation source, a spectroscope 2A of the near-infrared radiation, a lens 3A for collecting the near-infrared radiation, first optical fibers 10A for introducing the collected light to a body portion of the subject, second optical fibers 20A for receiving a resulting radiation emitted from the body portion, an optical fiber bundle 4A formed with the first and second optical fibers, a photo diode 5A as a detector of the resulting radiation, and an operation unit (not shown) for calculating the glucose concentration from outputs of the photo diode. A pattern of projection ends of the first optical fibers 10A and receiving ends of the second optical fibers 20A arranged on an end surface of the fiber bundle 4A is shown in FIG. 10. Each of the first and second optical fibers (10A, 20A) has a diameter of 500 mu m. A distance between centers of the projection end of the first optical fiber 10A and an adjacent receiving end of the second optical fiber 20A is 500 mu m.

As the light-emitting diode 1A, there are light-emitting diodes of InP system useable in first and second harmonic tone regions, and light-emitting diodes of GaAs system, or GaAlAs system useable in a third harmonic tone region. In this embodiment, a light-emitting diode of InP system having a center wavelength 1600 nm and a half-width of 160 nm is used. The spectroscope 2A is formed with a disc 30A, and a set of first, second and third interference filters (31A, 32A, 33A) disposed around a center of the disc. The disc 30A can be rotated by a motor 6A to select a required one from the first to third interference filters. The first interference filter 31A is used to provide a first near-infrared radiation having a center wavelength of 1585 nm and a half-width of 60 nm.The second interference filter 32A is used to provide a second near-infrared radiation having a center wavelength of 1530 nm and a half-width of 10 nm. The third interference filter 33A is used to provide a third near-infrared radiation having a center wavelength of 1680 nm and a half-width of 10 nm.

The center wavelength of the half-width of the first near-infrared radiation are determined according to the profiles of FIG. 11 which is a partially enlarged view of FIG. 3 obtained in the fourth embodiment. That is, the center wavelength of 1580 nm is a wavelength substantially corresponding to a maximum value of the regression coefficient which is observed within a first wavelength region of 1550 nm to 1650 nm having an absorption peak derived from OH group of glucose molecule. The half-width of 60 nm substantially corresponds to a wavelength region having 70 % or more of the maximum value of the regression coefficient within the first wavelength region.When the center wavelength and the half-width are determined by the procedure explained above, there is an advantage of simplifying the operation for determining the glucose concentration without degrading a prediction accuracy of the glucose concentration.

In place of the above-explained procedure, it is possible to determine the center wave length and the half-width of the first near-infrared radiation according to a profile indicative of a relation between wavelength and regression coefficient which is obtained by applying a glucose tolerance test to a subject, measuring absorption spectrums during the glucose tolerance test, and performing a multivariate analysis of the absorption spectrums. The center wavelength and the half-width are not limited to the values used in this embodiment. It is preferred to use the first near-infrared radiation having a center wavelength within a range of 1560 nm to 1640 nm and a half-width of 60 nm or less.

After absorption signals detected by the photo diode 5A is converted to absorbencies, the glucose concentration is determined by the use of a calibration line previously stored in the operating unit. It is preferred to determine the calibration line according to the method of any one of the aforementioned embodiments.

Prior to the multivariate analysis, it is preferred to perform a pretreatment of subtracting a value of wavelength within a near-infrared region from absorption signals or absorbencies. Alternatively, it is preferred to perform a pretreatment of dividing the absorption signals or the absorbencies by the wavelength value. In this embodiment, it is preferred to use as the wavelength value a wavelength selected from a range of 1540 +/- 10 nm or 1650 +/- 10 nm. In case of using a range of 900 nm to 1350 nm, in which harmonics of a second harmonic tone are observed, it is preferred to use as the wavelength value a wavelength selected from a range of 1060 +/- 10 nm or 1130 +/- 10 nm.

This application is based upon and claims the priority of Japanese patent Application No. 9-72150 filed in Japan on March 25, 1997, the entire contents of which are expressly incorporated by reference herein.

The features disclosed in the foregoing description, in the claims and/or in the accompanying drawings may, both separately and in any combination thereof, be material for realising the invention in diverse forms thereof.

LIST OF REFERENCE NUMERALS

1 halogen lamp
2 reflection mirror
3 lens
4 optical fiber bundle
5 flat-field type diffraction grating unit
6 array-type photo diode
7 A/D converter
8 operation unit
9 body portion
10 first optical fiber
20 second optical fiber
20a receiving end
20b receiving end
60 slit
1A light-emitting diode
2A spectroscope
3A lens
4A optical fiber bundle
5A photo diode
6A motor
10A first optical fiber
20A second optical fiber
30A disc
31A